TOXICOLOGY OF AFLATOXIN B_1, WARFARIN AND CADMIUM IN YOUNG PIGS

By

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To my son, Diego Andrés

To my wife, Clara Eugenia

Success has been the happiness
and togetherness in the achievement
of our goals. Your love and under-
standing make life worthwhile.

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The objectives of this experiment were to compare the toxic effects of aflatoxin B_1, a dihydrofuranocoumarin, and warfarin, a 3-(α-acetonyl-benzyl)-4 hydroxycoumarin; and also to determine whether an additive effect from either aflatoxin B_1 or warfarin occurs when cadmium is present in higher than normal levels in the diets of young pigs.

Thirty-six healthy weaned barrows, mixed breed, averaging 9 kg of body weight, were assigned at random to 6 treatment groups, 6 pigs per group: Group I - negative control; Group II - 0.2 mg/kg of aflatoxin B_1; Group III - 0.2 mg/kg of warfarin; Group IV - 83 µg/g of cadmium diet; Group V - 83 µg/g of cadmium diet plus 0.2 mg/kg of aflatoxin B_1; Group VI - 83 µg/g of cadmium diet plus 0.2 mg/kg of warfarin. Groups II, III, V and VI received 5 daily doses of the chemical in gelatin capsules during the fifth week of the experiment and the effects were followed for 10 days. Cadmium (Cd) was provided daily through the diets (given as cadmium chloride) during the 40 days of the experiment.

The body weight loss ($P < 0.0065$) in the aflatoxin B_1 group was associated with decreased feed consumption. Significantly lower values in serum total protein ($P < 0.0378$), alpha globulin ($P < 0.0133$), beta globulin ($P < 0.00119$), gamma globulin ($P < 0.05$) and plasma fibrinogen ($P < 0.0279$) were induced by aflatoxin B_1.

Significantly increased values of alkaline phosphatase ($P < 0.016$), sorbitol dehydrogenase ($P < 0.003$), and aspartate aminotransferase ($P < 0.05$) were determined 48 hours after initiation of the dosing with aflatoxin B_1 and correlated with hepatic fatty infiltration and vacuolation through all lobules.

Warfarin was more effective in producing earlier and higher values in prothrombin time ($P < 0.001$) and activated partial thromboplastin time ($P < 0.007$) than aflatoxin B_1 by the second day after initiation of dosage.

Depressed growth, feed consumption, feed efficiency, white blood cell counts ($P < 0.0056$), gamma globulin values ($P < 0.0018$) and an extreme microcytic hypochromic anemia were evident in the pigs consuming 83 µg/g Cd in the diet.

The Cd concentrations were highest in kidney, 42.9 µg/g next in liver, 7.92 µg/g and lowest in muscle tissues, < 0.22 µg/g, and correlated well with the loss of iron from the kidney ($P < 0.0001$) and liver ($P < 0.0001$) in pigs treated with 83 µg/g Cd diets.

Cadmium may have blocked the liver microsomal enzyme system and prevented the activation of aflatoxin B_1 to a toxic anticoagulant metabolite. On the other hand, that blockade prevented the inactivation of warfarin, enhancing its anticoagulant effects. This blockade was demonstrated through clinical signs, hematologic, electrophoretic and clinical chemistry changes, as well as gross and histopathologic lesions. It is concluded that there is an inhibitory effect on the toxicities of aflatoxin B_1 while there is an enhancing synergistic effect with warfarin when Cd is present in the diets of young pigs at 83 µg/g.

CHAPTER I

INTRODUCTION

Aflatoxins are a group of metabolites that vary in their toxic and carcinogenic potential (Edds, 1973; Cardeilhac and Nair, 1974; Newberne, 1973; Newberne and Butler, 1969).

The hepatotoxic properties of aflatoxin B_1 differ, depending on the dose and the duration of exposure (Newberne et al., 1979). Susceptibility to aflatoxins also appear to vary with species, breed, sex, age, nutrition, health status e.g., liver diseases and parasitic diseases (Osuna et al., 1977; Edds and Osuna, 1976; Edds, 1973).

The toxic anticoagulant properties of warfarin seem to also vary with those factors described above (Deckert, 1974; Coon and Willis, 1974; Bachman et al., 1977). Indeed, the anticoagulant rodenticides are more toxic when ingested daily over a period of 5 - 7 days. Multiple small doses are 5 - 100 times as toxic as the same single dose, depending on the species (Hatch, 1977).

Liver and renal function influence the duration and the intensity of response of oral anticoagulants (Deckert, 1974, Bachman et al., 1977).

Generally, cadmium concentrations are highest in the kidney, followed by the liver (Friberg et al., 1971). One

may suggest that research describing the interactions of cadmium with anticoagulants could be of importance since clinical complications of patients treated with coumarin derivatives have arisen with administration of other medications (Fasco et al., 1978).

The interactions associated with drug biotransformation are of special interest. Two types of interactions are possible, stimulation or inhibition of the drug metabolizing enzymes by other drugs, including the anticoagulants (Deckert, 1974).

Understanding the role of metals in the environment and their interactions with biological systems is becoming increasingly important to the overall understanding of their potential hazard to health (Brancato et al., 1976; Potts, 1965; Tsuchiya, 1969; Schroeder, 1965, 1967; Friberg, 1957). Cadmium is of interest in animal agriculture because of its toxicity; it is not an essential element (Neathery and Miller, 1976). Practices such as use of superphosphate fertilizer and municipal sewage sludge on farmland can make Cd available through plant products in livestock - human foodchains.

Another potential hazard with aflatoxin B_1 is its transmission of its metabolites into animal products such as tissues and milk. Aflatoxins or their metabolites that accumulate in milk or other tissues from food-producing animals may pose serious hazards to young animals or infants consuming such products (Edds, 1973; Cardeilhac et al., 1970).

The purpose of using cadmium supplemented diets in pigs was to determine whether animals may be predisposed to higher risk when exposed to moldy feed containing aflatoxin B_1 or other poisons such as rodenticides even if no histopathological liver lesions are detectable in pigs consuming such Cd diets.

The objectives of this experiment were to compare the toxic effects of aflatoxin B_1, a dihydrofuranocoumarin, and warfarin, a 3-(α-acetonyl-benzyl)-4 hydroxycoumarin; and also to determine whether an additive effect from either aflatoxin B_1 or warfarin occurs when cadmium is present in higher than normal levels in the diets of young pigs.

CHAPTER II

LITERATURE REVIEW

Aflatoxin B_1

The mycotoxicoses include a diverse group of diseases which affect several organ systems in various ways; therefore, clinical signs and lesions produced by them may vary with the particular toxin involved (Pier, 1973). The difficulty in diagnosing mycotoxicosis by clinical signs, lesions, history or other clinicopathologic methods makes it difficult to determine the incidence and seriousness of these diseases (Cardeilhac and Nair, 1974).

The occurrence of mycotoxicosis is associated with feeding field-harvested, as well as stored grains and in feeding concentrated rations when conditions of moisture, temperature and aeration are favorable to the toxigenic fungi (Edds, 1973; Pier, 1973).

The mycotoxins have been detected at levels in feed in the southeastern U.S. that result in liver damage and stunting in both swine and poultry. Both in the developed and developing countries, these two species afford the quickest way to provide proteins needed for human requirement (Edds and Osuna, 1976).

The aflatoxins, a group of closely related metabolites of Aspergillus flavus, are now well known to be potent

hepatotoxins possessing carcinogenic, mutagenic and teratogenic activity and can occur as natural contaminants in animal feeds, as well as in a wide variety of food material used for human consumption (Wogan, 1968; Edds, 1973; Newberne, 1973; Hatch, 1977).

The toxic properties of the aflatoxins differ, depending on the dose and the duration of exposure. Susceptibility to aflatoxins also appear to vary with species, breed, sex, age, nutrition, liver diseases and parasitic diseases (Newberne et al., 1969; Edds and Osuna, 1976; Osuna et al., 1977).

Aspergillus flavus and Aspergillus parasiticus, although present in most corn producing areas, have not been associated with high levels of aflatoxins in corn in the U.S. However, due to drought stress, corn insect damage and other environmental factors in 1977, the corn produced in the southeastern states, Alabama, Florida, Georgia, Mississippi and North and South Carolina had high levels, i.e., more than 200 - 400 ng/g aflatoxin B_1. It has been advised recently to blend corn with levels of aflatoxin B_1 up to 100 ppb with other corn to reduce the level of finished feeds to 20 ppb or less (Edds, 1979).

Several reports concerning the toxic effects on pigs of feeds naturally or experimentally contaminated with aflatoxins have been published (Gagné et al., 1968; Hintz et al., 1967a, 1967b; Hayes et al., 1978; Cysewski et al., 1968, 1978; Sisk et al., 1968; Harding et al., 1963; Annau et al., 1964; Monegue, 1977; Edds, 1979; Cardeilhac et al., 1970; Gumbmann and Williams, 1969; Loosmore et al., 1961; Burnside et al., 1957).

Earlier research in swine has indicated this species is more susceptible at a young age, 1 - 4 weeks old, and pregnant sows are more susceptible than mature cattle and sheep (Edds, 1979; Edds and Osuna, 1976). According to several sources, the acute LD_{50} of aflatoxin B_1 in young pigs is 0.52 mg/kg of body weight (Hatch, 1977) and older swine, 1.0 - 2.0 mg/kg of body weight (Edds, 1979). Dietary concentrations of aflatoxins that cause toxicosis (liver damage and/or death) in swine are: growing pigs, 0.41 µg/g or more for 12 - 24 weeks; pregnant sows, 0.3 - 0.5 µg/g for 4 weeks (Wogan and Shank, 1971; Buck et al., 1976; Edds, 1973; Ciegler, 1975; Hatch, 1977). In young pigs, as little as 51 ng/g aflatoxin in the feed eventually causes hepatic and blood chemical changes indicative of cell damage (Gumbmann and Williams, 1969).

Experiments reported from England (Allcroft and Carnaghan, 1963) on pigs weighing 40 kg and fed diets containing 140, 280, 410 and 690 ng/g aflatoxin B_1 indicated there was no growth suppression at the 140 ng/g level, but at 280 and 410 ng/g on a restricted ration, pigs showed growth suppression and at the 690 ng/g level, there were mild to severe clinical effects. Carnaghan and Crawford (1964) also reported that pigs on complete rations and at aflatoxin B_1 levels up to 410 ng/g showed no decreased rate of gains. At levels of 615 and 810 ng/g, there were decreased rate gains and decreased feed efficiency.

Hintz et al. (1967a) also investigated the toxic effects of aflatoxin exposure in young Duroc pigs, 13 - 14 weeks old, at 450, 615 and 810 ng/g levels in the feed for 117 days.

Rations containing 450 ng/g did not significantly affect gains or efficiency of feed conversion of growing-finishing pigs. Rations containing 615 ng/g aflatoxin B_1 resulted in slightly decreased weight gains, and rations containing 810 ng/g aflatoxin B_1 in greatly decreased weight gains and decreased feed conversion.

The growth rate and reproductive performance of 5 Duroc gilts and 5 Duroc boars fed a ration containing 450 ng/g aflatoxin B_1 were compared to the performance of 9 gilts and 7 boars fed standard rations. Aflatoxin B_1 had no significant effect on sperm motility, percent of live sperm or percent of abnormal sperm. Further, aflatoxin B_1 had no significant effect on the reproductive performance of the gilts (Hintz et al., 1967b).

Cysewski et al. (1968) produced acute aflatoxicosis in young pigs, 11.4 - 21.4 kg average body weight, by giving aflatoxin B_1 at 1.98 mg/kg. The pigs were depressed, with initial pyrexia followed by subnormal temperature. Shivering, muscular tremors and weakness were noticed at irregular intervals beginning at 6 - 12 hours. Fresh blood appeared in the feces at 24 hours. Altered liver function was detected at 3 hours and was marked at 6 hours. Serum glutamic-oxaloacetic transaminase and ornithine carbamyl transferase activities were elevated markedly after 6 - 9 hours. Leukocyte counts and prothrombin times were elevated after 12 - 24 hours. Chromatographs of urine eliminated at 3 - 9 hours showed metabolites of aflatoxin.

Sisk et al. (1968) produced experimental aflatoxicosis in 2 week old pigs by daily dosing for 23 days at 83.4, 166.8 and 333.6 µg/kg body weight of aflatoxin B_1. The smallest dose of aflatoxin did not produce clinical evidence of disease in the pigs, but mild hepatic changes were observed microscopically. The median dose level (166.8 µg/kg) was obviously toxic to young swine, and the lesions were similar to, but less severe, than those observed in swine given the largest dose level. The highest dosage resulted in reduced weight gains, depression, anorexia, hemoconcentration and icterus.

Cardeilhac et al. (1970) reported newborn pigs were stunted when they consumed milk of sows fed aflatoxin contaminated feed for the first 4 days after farrowing.

Hauser et al. (1971) reported outbreaks of hemorrhage in swine consuming feed containing 150 ng/g aflatoxin B_1. The normal handling of the pig led to massive subcutaneous hemorrhage. Addition of vitamin K_1, 2.2 mg/kg body weight, reduced the prothrombin times to normal and the hemorrhages ceased. Suttie (1973) suggested the absence of vitamin K or the presence of an "antivitamin" aflatoxin B_1 caused an increase in an antithrombin substance in the plasma. The albumin molecule is believed to form a complex with anticoagulants, binds thrombin and is a powerful antithrombin.

Neufville (1974) demonstrated that inclusion of aflatoxin B_1 at a level of 450 ng/g in the ration of 5.5 - 7.7 kg pigs for a 28 day feeding period produced significantly lower daily gains than a control group on standard ration.

Returning the pigs in the treated group to a normal ration resulted in their return to a normal growth curve. Addition of menadione or vitamin K to the ration for one group of the previously treated pigs improved their rate of gain over those on the ration without supplementation. The aflatoxin exposed pigs showed a significant prolongation in prothrombin time after the 28 day exposure period.

Monegue (1977) compared average daily gains in 2 groups, 7 pigs each, of Duroc-Yorkshire x Hampshire barrows and gilts receiving either a control ration or a ration naturally contaminated with aflatoxin B_1 at 400 ng/g level. The trial lasted 70 days. There was significant growth suppression in the group of pigs receiving the aflatoxin contaminated feed.

Levels of 100, 200 and 300 ng/g of aflatoxin B_1 in the ration did not significantly affect average daily gains, feed-consumption or efficiency, prothrombin times, or liver or kidney weights expressed as percent of body weights in young swine up to market weight (Monegue, 1977).

Few reports are available of confirmed instances of aflatoxicosis in swine under field conditions (Sippel et al., 1953; Loosmore et al., 1961; Hornby et al., 1962; Gibbons, 1965; Wilson et al., 1967; Smith et al., 1976; Hayes et al., 1978). The experimentally induced toxicity compared favorably to the naturally occurring disease reported by Sippel (1953).

Unseasonably cold weather apparently was a factor in initiating the onset of clinical signs and probably increased the severity of aflatoxicosis in pigs. Aflatoxin B_1 was found

in the plasma of 3 of 9 pigs examined at values ranging from 5.1 - 36.7 ng/ml. Aflatoxin B_1 was not detected in the kidneys but 0.12 ng/g was found in the pale yellow liver of one pig. Internal parasites were not found and gross lesions indicative of ascarid migration were not observed in the liver of any of the 5 pigs necropsied (Hayes et al., 1978).

Smith et al. (1976) reported 94 cases of swine aflatoxicosis over a 22 month period in North Carolina. Only aflatoxin B_1 with a mean value of 5.18 mg/kg (range 0.06 - 15 mg/kg) was found in the corn ingredient of the feed from these cases. The concentration of aflatoxin was higher in corn than in the feed (3.89 mg/kg), which suggested that the corn was the primary source of aflatoxin.

In swine, feeding diets containing 233 ng/g of aflatoxin did not lead to the presence of residues in the edible tissues (Booth, 1969). After 88 days of experimental feeding trials, significant alterations were evident in the blood from pigs receiving this level of aflatoxin (Gumbmann and Williams, 1969).

Biochemical changes accompanying the development of aflatoxicosis were observed in young pigs maintained for approximately 4 months on diets containing various concentrations of aflatoxin B_1 ranging from 2 to 810 ng/g. During the feeding period, serum glutamic-oxaloacetic transaminase, alkaline phosphatase, and isocitric dehydrogenase became elevated. Other factors in blood, namely plasma albumin, albumin:globulin ratio, non-protein nitrogen, urea nitrogen and adenine nucleotides (E_{260} values) all decreased. At autopsy, the concentrations in the liver of glutamic-oxaloacetic transaminase,

isocitric dehydrogenase, lipid vitamin A, glycogen and total nitrogen decreased as a function of increasing dietary aflatoxin (Gumbmann and Williams, 1969).

Early work by Harding et al. (1963) showed that 4 to 6 week old pigs fed a toxic groundnut meal, which presumably was contaminated with aflatoxin, responded with changes in serum enzymes and liver composition. Unfortunately, however, the aflatoxin content of this meal was unspecified. Besides, at levels of aflatoxin estimated to be 750 ng/g or more, it was found alterations in the electrophoretic patterns of pig serum proteins showed a relative decrease in the albumin, $alpha_1$, $alpha_2$ and beta globulins while gamma globulins appeared to be considerably increased (Annau et al., 1964). Acute intoxication by aflatoxin in pigs, leading to death between 24 and 72 hours, was accompanied by changes in serum enzyme levels and liver function indicative of marked hepatic damage (Cysewski et al., 1968).

Some of the biochemical changes that characterize developing aflatoxicosis are not unlike those generally associated with other hepatotoxins (Gumbmann and Williams, 1969). Marked increases in serum alkaline phosphatase, serum glutamic-oxaloacetic transaminase and isocitric dehydrogenase and other enzymes are known to result from poisoning by carbon tetrachloride, thioacetamide and dimethyl nitrosamine (Rees and Sinha, 1960; Rees et al., 1962) and have been related to progressive liver necrosis (Cornelius et al., 1959, 1963; Rees and Sinha, 1960; Musser et al., 1966). Alterations in liver

function as represented by changes in these enzymes occurred in pigs after feeding 450 ng/g aflatoxin or more, with the exception of alkaline phosphatase activity which became elevated at aflatoxin levels as low as 51 ng/g.

Both 4 and 10 week-old pigs, when fed rations containing high amounts of protein (20.6 - 17.0%) vs. low concentrations (14.1 - 11.4%), developed more serious signs and lesions at lower concentrations when exposed to 95 and 99 µg/kg body weight of aflatoxin respectively. These signs and lesions included stunting, icterus, with hepatic necrosis, hemorrhage, and bile duct hyperplasia, along with hydropic and fatty degeneration of hepatocytes (Edds and Osuna, 1976).

Gross hemorrhage occured in many parts of the body, especially in the ham areas. This increased pressure in the gluteal muscles led to ataxia, with animals presenting a dog-like sitting position with tachypnea or panting (Edds, 1979). The intestines and sometimes the abdominal cavity of pigs were filled wtih blood (Loosmore et al., 1961).

In general, it has been observed that the most apparent pathological changes occur in the liver in the presence of aflatoxicosis, involving a destruction of the normal hepatic architecture by a pronounced fibrosis and the proliferation of bile duct epithelium (Annau et al., 1964; Edds, 1973; Osuna et al., 1977).

Aflatoxins are absorbed from the intestinal tract and bound to serum albumin (Bassir and Bababunmi, 1973). Liver takes up aflatoxin from the blood. The rate of uptake depends on the aflatoxin structure and species. Within the hepatocyte,

aflatoxins may exert direct effects on nuclear DNA and RNA synthesis and on sex steroid binding sites on the endoplasmic reticulum. Aflatoxins may be excreted by the kidneys as non-fluorescent water-soluble sulfates and glucuronides (Hatch, 1977).

Aflatoxins are polycyclic, unsaturated compounds consisting of a coumarin nucleus flanked by an apparently highly reactive bifuran system on one side and either a pentenone (B toxins) or a 6-membered lactone (C series) on the other (Hatch, 1977).

Aflatoxin B_1 produced prolongation of blood clotting time in the rat. Results of *in vitro* studies indicate that the anticoagulant effect may be due mainly to competition with vitamin K in the production of prothrombin in the liver (Bassir and Bababunmi, 1972).

Attempts have been made to compare the blood anticoagulant properties of aflatoxin with those exhibited by 4-hydroxycoumarin in view of the similarities in the structure of the synthetic coumarins and the aflatoxins (Asao *et al.*, 1963). After a period of 3 hours, aflatoxin B_1 had prolonged the normal clotting time maximally in rats. The peak action of 4-hydroxycoumarin was reached after 48 hours in the same experiment (Bababunmi and Bassir, 1969). The thrombo-test technique used did not enable them to distinguish between various factors involved in blood clotting. Aflatoxin B_1 has been shown to be more effective than 4-hydroxycoumarin in prolonging the blood clotting time of rats (Bababunmi and Bassir, 1969).

Since aflatoxin B_1 inhibited liver protein synthesis in calves and acts as an antimetabolite of vitamin K, it would be reasonable to suggest that the formation of factors II, VII, IX, X as well as factor I is diminished (Osuna et al., 1977).

Aflatoxin B_1 requires metabolic action to elicit its carcinogenic effects (Campbell and Hayes, 1976). None of the known metabolites of aflatoxin B_1 are as active as the parent compound in the microsome-mediated assay. It is suggested that the active metabolite may be a 2,3 epoxide. A number of unsaturated compounds are readily epoxidized by the mixed function oxygenases of the liver microsomes (Garner et al., 1972). The epoxides of the carcinogenic polycyclic hydrocarbons are highly reactive, toxic and mutagenic (Cookson et al., 1971).

Aflatoxins are thought to be metabolized to highly reactive epoxides and phenolates (the furan system of aflatoxin is involved here) that can bind and theoretically interfere with nucleic acids and proteins (Ciegler, 1975).

The differences in species, as well as breeds within species, can be correlated with the differences in the rate of metabolism of aflatoxin B_1 (Paterson, 1973). The susceptibility of a specie is directly correlated with the hepatic transformation to aflatoxicol, but inversely related to its transformation to aflatoxin Q_1 or aqueous metabolites (Edds, 1979). Carnaghan et al., (1964) suggested aflatoxin may produce carcinomas in swine.

Warfarin

Swine are occasionally affected with some degree of bleeding disorder which may impair their performance or threaten their life. Outbreaks may involve high morbidity within a herd and occur spontaneously or in response to trauma, such as castration. Often, several outbreaks occur within a geographic area at a particular time of the year and associated with a common feedstuff (Osweiler, 1978).

Epizootic studies in recent years have characterized a syndrome in swine which occurs sporadically, sometimes involving large numbers of swine in a given geographic location or season of the year (Fritschen et al., 1970; Murher, et al., 1970; Osweiler et al., 1970). This syndrome is characterized by acute onset, with lethargy, anorexia, lameness, hemorrhagic diarrhea, and death. Gross lesions in swine dying of porcine hemorrhagic disease include anemia, hemarthrosis, subcutaneous hematomas, intramuscular hemorrhages and gastrointestinal bleeding. Blood may fail to coagulate for as long as 12 - 72 seconds. Clinicopathologic examination has been with finding of anemia, increased prothrombin time and normal thrombocyte and liver function (Osweiler, 1978).

Porcine hemorrhagic disease has been most prevalent in weanling swine consuming pelleted feeds which contain antibiotic or sulfonamide, or both drug combinations (Osweiler, 1978). Furthermore, hemorrhagic manifestation are ameliorated by administration of vitamin K (Fritschen et al., 1970; Murher et al., 1970; Osweiler et al., 1970). Examination

of coagulation factors revealed a deficiency of factor X and probable deficiency of factor VII; factors also decrease in toxicosis by coumarin anticoagulants (Osweiler et al., 1970; Coles, 1967; Deykin, 1970).

There has been a general feeling that antibiotic use in feeds may reduce intestinal synthesis of vitamin K to a point at which swine are more susceptible to a coumarin or anti-vitamin K agent in the diet (Osweiler, 1978). Research and clinical reports from human and veterinary medicine reflect conflicting opinions about the role of orally given anti-bacterial agents in the production or exacerbation of vitamin K deficiency (Osweiler, 1978).

Experimentally, some sulfonamides, especially sulfa-quinoxaline, have been found to alter the prothrombin complex status in rats and poultry. Some investigators have felt that this hypoprothrombinemic response was due to inhibition of bacterial vitamin K synthesis in the intestine and others have given evidence for the direct antagonism of vitamin K by sulfaquinoxaline in the liver (Green, 1966; Griminger, 1957, 1965; Griminger and Donis, 1960).

In the early 1920's, a hemorrhagic syndrome of cattle was observed in North Dakota and Canada. Observation of field cases established that the disease was associated with consumption of improperly cured or moldy sweet clover hay. Some ten years later, the bleeding was found to be due to a deficiency caused by a breakdown product of coumarin in moldy sweet clover. During the early 1940's, the Wisconsin Agricultural Experimental Station synthesized an active anticoagulant

principal called dicoumarol or bishydroxycoumarin (Buck et al., 1976).

Warfarin, 3-(αacetonylbenzyl)-4 hydroxycoumarin, an extensively used oral anticoagulant and rodenticide, functions as a vitamin K_1 antagonist via an unknown mechanism (Fasco et al., 1977). Generally, poisoning occurs when animals ingest baits intended for rats and mice. Swine, dogs and cats can be poisoned by eating rats or mice that were killed by these compounds (Hatch, 1977).

The anticoagulant rodenticides are structurally related to coumarin. All have the basic coumarin or indadione nucleus (Buck et al., 1976). Warfarin and congeners have anticoagulant properties that make them useful as rodenticides. This group of agents include warfarin (Warfarin, D-Con), pindone (Pival), diphacinone (Diphacin), chlorophacinone (Drat, Rozol), coumafuryl (Fumarin), naphthylindadione (Radione), coumatetralyl (Endox, Racumin) and other synthetic coumarin and indadione derivatives (Hatch, 1977; Buck et al., 1976).

Warfarin and congeners are odorless and tasteless; therefore, they do not tend to induce bait shyness in the rodent population. The poisons act slowly, over a period of about a week; therefore, the baits are carried to nesting places for consumption by other rodents (Hatch, 1977).

The anticoagulant rodenticides are more toxic when ingested daily over a period of 5 - 7 days. Multiple-dose toxicity may be 5 - 100 times the single dose toxicity, depending on the species (Hatch, 1977).

The anticoagulant rodenticides are a potential hazard to all animals and birds (Buck et al., 1976). Horses are resistant to warfarin, but toxicity data are lacking. Ruminants can tolerate a lot of warfarin; death occurs at a dosage of 200 mg/kg of body weight per day for 12 days. Dogs and cats are sensitive to warfarin. A single dose of 20 - 50 mg/kg can kill dogs and a single dose of 5 - 50 mg/kg can kill cats. Pigs are more susceptible to warfarin than are rats and mice. Ingestion of 0.05 - 0.4 mg/kg of body weight per day for 7 days can kill pigs (Clarke and Clarke, 1975; Buck et al., 1976). Rats and mice die after ingesting 1 mg of warfarin per kilogram of body weight per day for 5 days, or 50 - 150 mg/kg in a single dose (Hatch, 1977; Buck et al., 1976).

This resistance phenomenom has been a particularly useful tool for the vitamin K specialists (Deckert, 1974). On the other hand, genetic selection creates a serious rodent control problem, particularly in areas having strict eradication program. Cross resistance to other anticoagulants is frequent (though not absolute), but varies with different populations (Jackson and Kaukeinen, 1972). The newborn are particularly sensitive to oral anticoagulants (Goodman and Gilman, 1975).

Hermodson et al. (1969) and O'Reilly (1970, 1971) have proposed that warfarin resistance results from a mutation that causes the synthesis of a protein with a lowered binding affinity for both vitamin K and warfarin. This is supported

by the high vitamin K requirement in both warfarin resistant rats (Hermodson et al., 1969) and humans (O'Reilly, 1971) and the decreased ability of microsomes from resistant rats to bind warfarin in vitro (Lorusso and Suttie, 1972). The reduced binding of warfarin could be explained by the hypothesis of Bell and Caldwell (1973). The enzyme system which converts phylloquinone oxide to vitamin K_1 is found in the microsomal fraction (Zimmerman and Matschiner, 1972), while the high vitamin K requirement could be explained by an altered and less effective enzyme system in converting the oxide back to vitamin K_1 in the absence of warfarin. However, phylloquinone oxide was as effective as vitamin K_1 in stimulating prothrombin synthesis in hypoprothrombinemic resistant rats (Bell and Caldwell, 1973).

Warfarin is widely employed as a rodenticide and as a therapeutic drug for the treatment of such coagulation disorders as thrombophlebitis, pulmonary embolism and myocardial infarction. Human and rodent resistance to warfarin has been encountered, and clinical complications have arisen as a consequence of its administration with other medications (Fasco et al., 1978).

The coumarin anticoagulant drugs, as exemplified by warfarin, are still used with considerable caution due to the potential irregularities in the degree of anticoagulant effect. The fundamental reasons for the wide variation in daily maintenance doses of warfarin for the general population have not been documented (Coon and Willis, 1970); and the mechanism of action of warfarin

is only partially understood (Nelsestuen and Suttie, 1973; O'Reilly, 1972; Caldwell et al., 1974).

Thus, patient monitoring for coagulation irregularities is required and is usually done using the one-stage prothrombin time technique (Quick, 1972) and associated prothrombin complex activity (PCA) (Loeliger, 1972). PCA also has been used in describing the pharmacodynamics of warfarin (O'Reilly et al., 1963). However, warfarin inhibits the synthesis of activity of the individual vitamin K-dependent clotting factors II, VII, IX and X for which the PCA rate of synthesis is only an approximation (O'Reilly and Aggeler, 1970).

Several factors may contribute to the instability of anticoagulant therapy. However, the primary variables are clearly the amounts of coumarin and vitamin K available to the human liver each day (Udall, 1968). A precise dose of coumarin drug is administered and intestinal absorption of warfarin is known to be complete (Clatanoff et al., 1954; Shapiro and Ciferri, 1957). Plasma levels of warfarin were found to be identical following oral and intravenous administration (O'Reilly et al., 1963). No warfarin was found in stools after large oral doses (400 mg). On the other hand, the amount of vitamin K available to the liver each day is unknown (Udall, 1968).

Several combinations of large doses of warfarin and vitamin K were administered to two human subjects in an effort to obtain a stable course of substained anticoagulant therapy. Favorable results were obtained, but only when vitamin K_1

phytonadione was administered by subcutaneous injection (Udall, 1968).

Warfarin was introduced into clinical anticoagulant therapy in 1952. Since 1958, sodium warfarin has been the leading anticoagulant in clinical practice. It is estimated that about 85 percent of the oral anticoagulation therapy in the U.S. is via sodium warfarin (Coumadin). However, the range between efficient therapy and undue hemorrhagic risk may vary greatly from one patient to another; the need for carefully individualized treatment and frequent observations has long been stressed (Deckert, 1974). This wide variability in responsiveness is due to numerous factors, most of which were recognized by Link (1959). A brief summary of some of the variables follows:

1. There are wide species differences in hypoprothrombinemic response induced by the agents, and these vary further with the age and sensitivity of each individual.

   These species differences include not only pharmacokinetic parameters, but also the nature of metabolic end products. They are so marked that considerable caution is necessary in extrapolating the effects in one or even several animal species to man (Deckert, 1974).

   A very interesting aspect of this variability is represented by individuals that are apparently resistant to the coumarin anticoagulant drugs.

Resistance to anticoagulant rodenticides by Norway rats, especially in Northern Europe, was discovered in the early 1960's, and O'Reilly and associates (1964) pointed out that the extradorinary tolerance to these drugs observed in certain patients is unlikely to be the tail end of a normal continuous frequency distribution. The heredity transmission of this resistance is determined by an autosomal gene. Heterozygous rats have a 20 - 25 fold tolerance to warfarin on a mg/kg basis, and the first reported resistant human patient required 145 mg/kg warfarin per day as compared to 7 ± 3 mg for normal patients (Deckert, 1974).

2. Liver and renal function influence both the intensity and the duration of response.

The influence of disease states upon the disposition and pharmacology of drugs and, consequently, the rational selection of drug dosing regimens is receiving ever increasing attention in the pharmacological and medical literature (Bachmann et al., 1977).

Among physiologic factors, hepatic disorders are the most important. Malabsorption of the drugs naturally would decrease the response (Deckert, 1974). Diminished doses of drugs are frequently advised for patients with hepatic disease receiving drugs eliminated largely by way of hepatic metabolism

(Bachmann et al., 1977). However, the elimination half-lives of dicoumarol appear to be unchanged in patients with liver disease (Wilkison and Schenker, 1975).

For some drugs whose elimination is directly related to creatinine clearance, it is possible to make appropriate dosage adjustments for renally impaired patients on the basis of routine evaluations of renal function (Bachmann et al., 1977). Warfarin represents an acidic, anionic drug whose extent of plasma protein binding is diminished in patients with renal dysfunction (Bachmann et al., 1976). However, it appears that patients with renal dysfunction do not possess an increased susceptibility, either pharmacokinetic or pharmacologic, to the hypoprothrombinemic effect of warfarin (Bachmann et al., 1977).

Unlike man, who excretes warfarin and its metabolites predominantly in the urine, the rat excretes a significant amount by the fecal route (Wong et al., 1978). This difference prompted an investigation of the interaction in an animal closer to man in its response to the drug (Wong et al., 1978). The guinea pig, like man, excretes warfarin and its metabolites predominantly in urine with about 43 percent of the warfarin dose being excreted into the bile in 12 hours after injection. A significant

portion of this consisted of unconjugated warfarin and its hydroxy-metabolites (Wong et al., 1978).

Salicylates displaced serum protein bound warfarin in vitro. Salicylates enhanced the biliary elimination of warfarin only at high concentration by displacing some of that bound to plasma protein. This facilitated uptake of warfarin by liver, where it was metabolized. In vitro, salicylates compete with warfarin for protein binding sites, thereby increasing the pool of unbound drug (Wong et al., 1978). This effect of salicylate did not modify the hypoprothrombinemia produced by warfarin (Wong et al., 1978).

In humans, large doses of salicylates have been associated with bleeding problems, especially in patients receiving oral anticoagulants. This may be due to direct gastric irritation, suppression of platelet function or hypoprothrombinemia (Goodman and Gilman, 1975).

It is of interest that one of these investigations identified salicylic acid as a metabolite of warfarin, since salicylates frequently are implicated in hemorrhagic complications. This finding was no real surprise, however, since chemical degradation of coumarin compounds usually lead to salicylic acid as the major product (Deckert, 1974).

Patients receiving anticoagulants may develop spontaneous intramural hematomas in the bowel which cause intestinal obstruction. The diagnosis is based primarily upon a history of anticoagulant therapy, bowel obstruction and a prolonged prothrombin time. A majority of patients also have other hemorrhagic manifestations; hematuria is the most common. The commonest site of abnormal bleeding is the urinary tract and the most frequent cause of death is intracerebral hemorrhage or gastrointestinal bleeding (Crisler et al., 1970).

3. The general nutritional status of the recipient influences both the intensity and the duration of response.

Fasting generally enhances it in all species, as do various vitamin and protein deficiencies. Vitamin K plays an obvious role, since its effect is competitive with the anticoagulants (Deckert, 1974).

The presence in the large bowel of certain antimicrobial drugs or other agents may alter the normal microflora, an important source of vitamin K (Goodman and Gilman, 1975).

The role of vitamin C remains somewhat mysterious (Deckert, 1974). Scurvy enhances or prolongs the oral anticoagulant response (Goodman and Gilman, 1975). Capillary fragility is associated with this enhancement (Deckert, 1974).

Pregnant and lactating women tend to be somewhat tolerant to the action of anticoagulant drugs. Although fetal deaths and other severe outcomes have been correlated with the use of anticoagulant drugs during pregnancy, the extent of excretion of the drug via the milk of lactating mothers has not been examined carefully (Deckert, 1974; Goodman and Gilman, 1975).

The coumarin anticoagulants pass the placental barrier. Severe hypoprothrombinemia with cerebral injury has been reported in the newborn infants whose mothers receive dicoumarol antepartum. The newborns are particularly sensitive to oral anticoagulants (Goodman and Gilman, 1975). Lactating animals and animals given lactogenic hormone may be resistant (Levine, 1975).

Warfarin and dicoumarol are subject to the same major biotransformation pathways (Buck et al., 1976). Dicoumarin is hydroxylated by hepatic enzymes to inactive compounds, which are excreted in the urine. Their metabolites have no anticoagulant effect. Small quantities, if any, appear unchanged in the urine; they may cross the placenta or be excreted in the milk (Hatch, 1977).

4. Drug interactions may alter the magnitude of the blood clotting effect.

Publications on drug interactions with anticoagulants are probably more numerous than for any

other class of drugs, not only because such interactions are common, are usually therapeutically important and occasionally life-threatening, but also because they are relatively easy to detect and analyze (Koch-Weser and Seller, 1971). Such alterations in anticoagulant action may arise via several different mechanisms, including altered vitamin K bioavailability, direct or indirect effects on clotting factor concentrations, or other aspects of the clotting mechanism, altered absorption, competitive binding to plasma proteins and changes in drug metabolism (Deckert, 1974).

The interactions at the site of drug biotransformation are of special interest. The two types of interactions possible here are stimulation or inhibition of the drug-metabolizing enzymes by either the other drugs or the anticoagulants (Deckert, 1974).

Drugs known to increase the displacement of warfarin from plasma binding sites include phenylbutazone, sulfonamides and adrenocorticosteroids (Buck et al., 1976). This action can result in a temporarily large increase in the plasma of free anticoagulant, since, for example, 97 percent of warfarin is bound at therapeutic concentrations. This results in a prolongation of the prothrombin time (Goodman and Gilman, 1975). The half life of the anticoagulant is shortened, and a new steady

state is eventually established. Enhancement of anticoagulant effect is thus temporary, but hemorrhagic catastrophies have resulted when this complication has been overlooked (Goodman and Gilman, 1975).

Corticotropin and adrenocorticoids have been reported to cause severe hemorrhage when given with oral anticoagulants (Cauwenberger and Jaques, 1958). This is rather puzzling, since thrombotic episodes are stated to occur more frequently in patients on steroid therapy, and oral anticoagulants have been advised in the prophylaxis of such complications (Rawls, 1955). Administration of ACTH, steroids or thyroxin may increase receptor site affinity for anticoagulants (Hatch, 1977). When barbiturate is administered concurrently, the half-life of coumarin anticoagulant is reduced (Goodman and Gilman, 1975). Anticoagulant metabolism, especially with regard to drug interactions, suggests that hepatic microsomal mixed function oxidase (namely, cytochrome P 450) is solely responsible for these biotransformations. On the other hand, several observations suggest that cytochrome P 450 levels may not always be rate-limiting for warfarin metabolism (Deckert, 1974), although it takes part in this reaction.

Laliberte *et al.* (1976) showed that fasting and immobilization stress both caused prolonged

prothrombin times in rats receiving warfarin, but had no effect on control animals. The authors attributed this difference to the displacement of albumin-bound warfarin by increased free fatty acids present in the blood during fasting and stress. However, stress may have been a minor factor in the chronic study (Vanieri and Wingard, 1977).

Serial blood samples were obtained from each rat for 50 - 70 hours after an acute dose of warfarin or for 120 hours after a chronic loading dose plus 12 hour maintenance doses of warfarin in order to compare the time course activities and rate of synthesis of activities for separate clotting factors II, VII, IX and X and to relate the rate of synthesis and activity of each factor to the plasma concentration of warfarin (Vanieri and Wingard, 1977). During periods of dosing with warfarin (acute dosing), factors VII and X activities and rates of synthesis of activity showed large rapid changes, while factors II and IX responded more slowly. As the warfarin concentration diminished, the factor X rate of synthesis and activity appeared to exceed predrug values in all rats. During chronic dosing with warfarin, the factor II activity and rate of synthesis of activity was depressed the most (Vanieri and Wingard, 1977).

The mechanism of action of coumarin anticoagulants is of great interest because of their therapeutic and rodenticide values and for the insight into the mode of action of vitamin K (Bell et al., 1972; Buck et al., 1976). It has been generally accepted that the coumarin drugs and vitamin K compete for an unknown active site but the effect of warfarin on the metabolism of vitamin K_1 has suggested an entirely different mechanism of action (Bell et al., 1972).

Warfarin had little effect on the subcellular distribution of vitamin K_1 (Bell and Matschiner, 1969) but caused the accumulation of phylloquinone 2,3-epoxide, a metabolite of vitamin K_1 (Matschiner et al., 1970). This oxide stimulated prothrombin synthesis in vitamin K deficient rats but was ineffective in warfarin-treated animals (Bell and Matschiner, 1972). Evidence that the oxide is a competitive inhibitor of vitamin K_1 in warfarin-treated rats suggested that warfarin exerts its effect by increasing the ratio of oxide to vitamin K_1 in the liver and the oxide inhibits the activity of the vitamin by competing with it for an active site (Bell et al., 1972).

In other words, phylloquinone oxide had approximately the same activity as vitamin K_1 in vitamin K deficient rats but was ineffective in those treated with warfarin because the activity of the oxide was due to its conversion to vitamin K_1, a reaction inhibited by warfarin in vivo (Bell and Matschiner, 1970).

A regulatory protein with binding sites for vitamin K and warfarin has been proposed by Olson et al., (1969); Hermodson et al., (1969); and O'Reilly (1970). Bell et al., (1972) suggest that if such a protein exists, vitamin K and the oxide compete for binding sites rather than the vitamin and coumarin drugs. If the hypothesis concerning the mechanism of action of warfarin is correct, resistance to the anticoagulant might occur by a mutation which renders the conversion of phylloquinone oxide to vitamin K_1 no longer sensitive to warfarin inhibition (Bell et al., 1972).

It is also proposed that warfarin is unable to counteract large doses of vitamin K_1 because the oxide:K_1 ratio does not reach an inhibitory level (Bell et al., 1972). Bell and Matschiner (1969) found that vitamin K_1 and phylloquinone oxide were competitive antagonists, while the relationship between the vitamin and coumarin anticoagulant is not a simple one (Lowenthal and MacFarlane, 1964; Lowenthal and Birnbaum, 1969).

It was suggested that DT-diaphorase serves to convert oxidation products of vitamin K back to the vitamin and a decrease in its activity would lead to vitamin K deficiency in resistant and coumarin-treated normal rats (Bell and Caldwell, 1973).

Vitamin K_1 or Phylloquinone or Phytonadione

Vitamin K is a dietary principle essential for the normal biosynthesis of several factors required for clotting of blood (Cohn, 1975).

In the late nineteen-thirties, vitamin K was discovered as a dietary antihemorrhagic factor needed to maintain plasma levels of prothrombin, and in the early forties, the coumarins were identified as indirect anticoagulants which functioned by antagonizing the action of the vitamin (Suttie, 1973).

The early investigations described above showed that vitamin K is a fat-soluble substance present in hog liver fat and in alfalfa. Subsequently, it has been demonstrated that the vitamin is concentrated in the chloroplast of plant leaves and in many vegetable oils. The feces of most species of animals contain large amounts of the vitamin, which is synthesized by the bacteria in the intestinal tract (Pennock, 1966; Cohn, 1975).

The K group of vitamins consists of two major chemical forms, both fat-soluble and widely distributed in nature:

1. Vitamin K_1 now known as phylloquinone, which is exclusively produced by plants.
2. Vitamin K_2, including a series of compounds termed collectively the menaquinones, and produced only by microorganisms (Pennock, 1966; Barkhan and Shearer, 1977).

The natural vitamin K_1 and menadione are lipid soluble substances. It is possible to make active water-soluble derivatives of menadione by forming the sodium bisulfite salt or the tetrasodium salt of the diphosphoric acid ester. These compounds are converted in the body to menadione (Cohn, 1975).

The only established function of vitamin K_1 and menaquinones in man and other mammals is the synthesis by the liver of four plasma coagulation factors, i.e., factor II (prothrombin), factor VIII (proconvertin), factor IX (plasma thromboplastin component, christmas factor) and factor X (Stuart factor) (Barkhan and Shearer, 1977; Zieve and Solomon, 1969). The K vitamins bring about the carboxylation of the glutamic acid residues at the NH_2-terminal end of the polypeptide chains of these clotting factors, which thereby acquire the property of binding calcium ions and thus become biologically active in the coagulation mechanism (Stenflo et al., 1974); but the biochemical mechanism by which the K vitamins bring about these molecular changes is unknown (Barkhan and Shearer, 1977).

There seems good evidence to indicate that alterations in the vitamin K status of animals control the rate of synthesis of prothrombin rather than its rate of degradation, and the vitamin-sensitive step is not associated with release of prothrombin from the liver (Suttie, 1973). More recently, it has been shown that the appearance of plasma prothrombin is preceded by a transient increase of prothrombin in liver microsomal preparations (Shah and Suttie, 1972).

The current situation is, therefore, one where investigators in this field generally agree that the control of prothrombin biosynthesis by vitamin K is exerted at some post-transcriptional site but disagree about the actual location of the control site (Suttie, 1973).

Over the last few years, a number of investigators have observed that the rapid burst of prothrombin which is seen following administration of the vitamin is only partially inhibited by a dose of cyclohexamide which will almost completely block the incorporation of labelled amino acids into plasma and tissue proteins. These observations suggest that protein synthesis is not involved in the vitamin-sensitive step (Suttie, 1973).

The general hypothesis that a precursor protein was involved was given added support when Shah and Suttie (1971) presented data based on the administration both of radioactive amino acids and of vitamin K to hypoprothrombinemic rats which indicated that the prothrombin formed in the presence of cyclohexamide did not contain newly synthesized protein, and that it must have been derived from a previously existing precursor pool (Suttie, 1973).

The results of these experiments, which clearly suggest a liver precursor to prothrombin, are not in agreement with reports by Olson and co-workers that the vitamin acts by stimulating de novo synthesis of prothrombin. To explain the apparent cyclohexamide insensitivity of the prothrombin response, it has been postulated that vitamin K is able to reverse the effect of cyclohexamide on those ribosomes making prothrombin, but not the rest of the ribosome population (Suttie, 1973).

The hypothesis that there may be a precursor to prothrombin has been strengthened by the observation of new

proteins in the plasma of animals given coumarin anticoagulants. These new proteins were first isolated from bovine plasma of animals given dicoumarol as the concentration of prothrombin decreased. It cross-reacted with antibody prepared against bovine prothrombin and showed complete immunological identify with prothrombin. The amino acid composition, carbohydrate composition, amino and carboxyl terminal amino-acids and molecular weight of the two proteins appeared to be identical. The abnormal prothrombin could be electrophoretically separated from prothrombin in the presence, but not in the absence, of calcium ions, and in contrast to normal prothrombin, it did not adsorb to barium salts (Suttie, 1973).

Another currently interesting aspect of the vitamin K problem has been the discovery of a new metabolite of the vitamin in the liver. In a series of papers, Bell and Matschiner and their co-workers have found that in warfarin treated rats, much of the liver vitamin is present as the 2,3 epoxide of the vitamin. They have postulated that the action of the coumarin anticoagulants is mediated through the build up of this 2,3 epoxide which then acts as an inhibitor of the vitamin. There is now considerable evidence that the synthesis of prothrombin and the related vitamin-K dependent clotting factors is linked to a metabolic cycle in which vitamin K is oxidized and reduced via its 2,3 epoxide metabolite. Since all the studies on this aspect of vitamin K metabolism have so far utilized phylloquinone (vitamin K_1),

the cycle has been termed the K_1-epoxide cycle (Shearer et al., 1976).

This cycle depends upon at least two enzymes which have been partially characterized as phylloquinone epoxidase (Willingham and Matschiner, 1974) and phylloquinone epoxide reductase (Matschiner, Zimmerman and Bell, 1974; Zimmerman and Matschiner, 1974). Matschiner et al. (1974) have shown that warfarin inhibits phylloquinone epoxide reductase in vitro, thus explaining their earlier findings that in vitro large amounts of phylloquinone 2,3 epoxide accumulated in the livers of warfarin-treated rats (Matschiner, et al., 1970). They have further proposed that the vitamin K-dependent step in the synthesis of the clotting factors is linked to the epoxidation of the vitamin (Willingham and Matschiner, 1974) and the role of the reductase is to regenerate vitamin K (Zimmerman and Matschiner, 1974).

In clinical practice, where oral anticoagulants are widely used for the prophylaxis and treatment of thrombo-embolic disease, all the current drugs are structurally related to either coumarin or indanedione. Representative drugs from each of these two groups have so far all been found to cause the accumulation of phylloquinone 2,3 epoxide in the liver of rats by inhibiting reduction of the epoxide to the vitamin (Ren, Laliberte and Bell, 1974; Sadowski and Suttie, 1974; Ren et al., 1977). In humans, others had previously reported that phylloquinone 2,3-epoxide also

accumulated in the plasma of warfarin-treated subjects (Shearer, McBurney and Barkhan, 1973).

Apart from measurements of the accumulation of phylloquinone 2,3-epoxide in the plasma, the isolation and characterization of the normal metabolites of phylloquinone from human urine (Shearer and Barkhan, 1973; Shearer, McBurney and Barkhan, 1974) provides a further technique to study the effects of warfarin on the metabolism of phylloquinone. It has been shown that warfarin alters the pattern of conjugates in urine (Shearer et al., 1974).

The log dose-response relationships between the dose or plasma concentrations of warfarin and the changes in the metabolites of phylloquinone in plasma and urine are typical of those to be expected for a drug-receptor interaction, and suggest that warfarin interferes with a receptor involved in the normal metabolism of phylloquinone. The identification of phylloquinone epoxide as a major metabolite which accumulates in the plasma of warfarin-treated subjects suggests that this receptor is phylloquinone epoxide reductase, an enzyme that normally converts phylloquinone epoxide into phylloquinone (Shearer et al., 1977).

In the rat, the inhibition of phylloquinone epoxide reductase by warfarin prevents the normal regeneration of phylloquinone via vitamin K_1-epoxide cycle and causes phylloquinone epoxide to accumulate in the liver (Matschiner et al., 1973). In man, the progressive inhibition of this enzyme by increasing doses of warfarin would account for both the

increasing concentration of phylloquinone epoxide in the plasma, and as more of the vitamin was trapped as the epoxide, for the decreased excretion of the normal aglycones of phylloquinone in the urine, metabolites I and II. The inverse relationship between the accumulation of phylloquinone epoxide in the plasma and the urinary excretion of metabolites I and II also suggest that these metabolites in plasma and urine were derived from the same metabolic pool of phylloquinone (Shearer et al., 1977). The effect of warfarin blocking the urinary excretion of the normal metabolites I and II was accompanied by a corresponding increase in the excretion of at least 3 abnormal metabolites denoted W_1, W_2 and W_3 (Shearer et al., 1977).

Patients with coelic disease and those with pancreatic insufficiency absorbed substantially less vitamin K_1 than normal subjects, and patients with complete obstruction of the bile duct showed virtually no absorption (Shearer et al., 1974).

Cadmium

In recent years, it has become apparent that cadmium concentration in humans is increasing as a consequence of the rapid expansion in industrial technology that has introduced into the environment increasing quantities of cadmium (Brancato et al., 1976). There is evidence that occupational and environmental exposure to cadmium has caused serious disease states, such as kidney damage (Potts, 1965), emphysema

(Friberg, 1957), osteomalacia (Tsuchiya, 1969) and arteriosclerotic heart disease (Schroeder, 1965, 1967).

Cadmium (Cd) is of interest in animal agriculture because of its toxicity; it is not an essential element (Neathery and Miller, 1976). Cd, which is usually obtained as a by-product of zinc mining and refining, is widely used in plastics, tires, pigments, batteries and plating alloys (Ammerman et al., 1973). Cadmium is present in water, grains, dairy products and vegetables (Koller et al., 1975).

Cadmium has been found in cigarette smoke (Menden et al., 1973), and a significant increase of cadmium was present in the kidney and liver of humans that had emphysema and carcinoma of the lungs (Flick et al., 1971).

The extensive investigations performed in the U.S. on the storage of metals in human organs have disclosed many interesting facts. While the concentrations of certain essential metals in the body remain largely the same throughout life, a number of nonessential metals, such as cadmium, have been found to increase steadily from birth up to 40 - 50 years of age, especially in the kidneys (Piscator et al., 1972). The biological half-life of Cd is estimated to be 10 - 25 years, and therefore, the metal tends to accumulate in the body (Koller et al., 1975). It has been calculated that the body of the "standard American" contains about 30 mg of Cd, one third of which is located in the kidneys (Piscator et al., 1972).

The kidneys and other tissues of infants do not contain Cd in a detectable quantity (Schroeder, 1965, 1967), whereas it is detectable in the corresponding adult tissues (Kendrey and Roe, 1969). It has been suggested that the steady accumulation of Cd in the kidneys with age is responsible for hypertension and atherosclerotic heart disease (Schroeder, 1967). The fact that the fetal kidney does not undergo glomerular filtration may explain the low levels at birth. As more nephrons become active in early life, more Cd becomes stored in the cortex as Cd-binding protein (CD-BP). The primary period of rapid renal accumulation is said to be in the first three years of life, during which time the Cd levels rise some 200 times (Fassett, 1975). The U.S. allows 0.01 µg/g of cadmium in drinking water (Koller, 1975).

Proteinuria is considered to be the first sign of tubular dysfunction and is said to occur when renal cortical levels of Cd reach about 200 ppm (wet weight) compared to normal levels of about 50 ppm in adults (Friberg et al., 1971). Loss of body weight and increased urinary excretion of protein, alkaline phosphatase and acid phosphatase were found to be early warning signs suggestive of renal injuries by Cd intoxication in rabbits (Nomiyama et al., 1973).

Histochemical study of enzymatic distribution in the healthy human kidney revealed that glomeruli are rich in acid phosphatase, proximal tubules in alkaline phosphatase, distal tubules in aspartate aminotransferase, alanine aminotransferase, glutamic-pyruvic transaminase and lactate

dehydrogenase (Wachstein, 1955; Bonting et al., 1960a, 1960b, 1960c; Pollak and Mattenheimer, 1962; Mattenheimer, 1968). Once a drug has destroyed renal cells, their cellular enzymes may be released into urine. Therefore, proximal tubular injury might be detected by a significant increase in urinary excretion of alkaline phosphatase accompanied by an increase in aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase. Stop-flow analysis, performed at the end of successive cadmium administrations, revealed proximal tubular dysfunction, as well as glomerular and distal tubular dysfunction (Nomiyama et al., 1973).

Generally, highest Cd concentrations are in kidney, followed by liver (Friberg et al., 1971). However, initially with low intake, liver may have more Cd than kidney (Burch and Walsh, 1959). The liver, which is much larger than kidneys, contains more total Cd (Neathery and Miller, 1975). Of the total body burden of radioactive Cd, the kidney and liver contained 73 percent in goats and 42 percent in cows 14 days after oral dosing. Tissues and contents of gastrointestinal tract contain a substantial amount. Although they represent a large proportion of body mass, muscles contain a relatively small part of the total body Cd. Thus, muscle tissue is well protected from ingested Cd (Neathery and Miller, 1975).

The main routes of Cd absorption are the respiratory and gastrointestinal tract (Neathery and Miller, 1975). Little Cd penetrates the skin (Friberg et al., 1971).

Usually, the greatest industrial hazard for humans and animals is from pulmonary absorption of Cd. About 10 - 40 percent of inhaled Cd is retained (Friberg et al., 1971) with the exact amount influenced mainly by mucociliary and alveolar clearance in the lungs (Neathery and Miller, 1975). Absorption of dietary Cd from the gastrointestinal tract is very low (Friberg et al., 1971; Miller, 1973). Using retention data, net ^{109}Cd (radioactive Cd) absorption was 0.35 percent for goats (Miller et al., 1969) and 0.75 percent for cows (Neathery and Miller, 1975). Cd absorption may be increased somewhat with low protein or low calcium diets, with certain diseases or when fecal elimination is blocked (Neathery and Miller, 1975).

Response to cadmium is affected by different factors such as sex, age, stress, health, nutrition and pattern of exposure. Thus, some sectors of the community may be more at risk than others (Webb, 1975). Cd interferes with metabolism of iron (Fe), calcium (Ca), zinc (Zn), as well as copper (Cu). Thus, Cd may be a greater hazard in areas where anemia is endemic, or in children and pregnant women in whom iron and calcium intakes may be inadequate (Six et al., 1972). Male rats receiving 125 µg/g Cd in the diet died in about 50 days while the majority of the females on a similar diet survived a very much longer time (Johns et al., 1923).

The effect of long-term Cd treatment upon distribution of the metals, copper, iron and zinc has been compared in various organs of the male and female rats. The renal accumulation of cadmium was similar in both sexes without a plateau

being reached. In contrast, the hepatic accumulation of cadmium was higher in the female than in the male rat and a plateau was observed after 30 - 35 weeks of dietary Cd treatment. Accumulation of cadmium was associated with an increased zinc concentration in the liver and an increased copper concentration in the kidney; these increases were correlated with increases in liver and kidney metallothioneins induced by cadmium. A loss of iron from the liver and kidney was also observed following dietary cadmium treatment and involved mainly loss of iron from ferritin (Stonard and Webb, 1976).

A metal-binding protein, metallothionein, has been isolated by Kägi and Vallee (1961) and by Pulido et al., (1966) from horse and human kidney cortex respectively. Since then, several investigators have described the phenomenon in chickens (Weser et al., 1973), mice (Nordberg et al., 1971), rats (Weser et al., 1973; Chen et al., 1975) and rabbits (Nordberg et al., 1972).

Pulido et al. (1966) have shown that human kidney metallothionein has a molecular weight of 10,500, and this protein contains 2.6 percent Zn, 4.2 percent Cd, 0.5 percent Hg and 0.3 percent Cu by weight. From these data, it may be deduced that in the physiological system, metallothionein can associate with all three elements of the II-B subgroup of the periodic table, as well as with copper (Shaikh and Lucis, 1972).

Since its discovery, metallothionein has been identified in the cytosolic fraction of liver, kidney and other

parenchymatous tissues of a wide variety of animal species and also in microorganisms. However, there is substantial variation in its natural abundance. For instance, a single human liver may contain as much as 200 mg. On the other hand, most experimental animals have lower natural levels of metallothionein (Kojima and Kägi, 1978).

The demonstration that liver and kidney of the horse contain metallothioneins of similar composition but of different ratios of zinc to cadmium, indicates that the metal selectivity of these proteins is determined primarily by physiological circumstances rather than by their polypeptide structure (Kojima and Kägi, 1978). Furthermore, using inhibitors of protein synthesis, it has been revealed that Cd regulates the production of renal metallothionein at the "translation" level and that of the hepatic protein at the "transcription" level (Shaikh and Smith, 1976).

In all probability, metallothionein is produced by various tissues to bind the toxic Cd ions. In the absence of a well-defined excretory mechanism, the element is retained in the tissues, tightly bound to metallothionein. A small fraction of Cd is slowly released into the blood. Most of it is taken up by the kidney and very little is excreted in the urine. This explains the long half-life of Cd and the age-dependent increase in the concentration of Cd in kidney (Friberg et al., 1974).

The detoxification function of metallothionein is supported by reports that chronic low level exposure of

experimental animals to Cd, which results in high tissue levels, does not seem to produce adverse effects (Nordberg et al., 1971; Nordberg, 1971). It is probable that toxic effects develop only under conditions where exposure exceeds the threshold (metallothionein-synthesizing ability) of liver, kidney and other tissues. The non-metallothionein bound Cd competes and displaces essential cations from enzymes and nucleic acids, thus affecting their physiological function and causing toxicity (Vallee and Ulmer, 1972; Shaikh and Smith, 1976).

Copper and/or zinc-thioneins also occur normally in variable amounts in the liver, kidneys and intestines of different mammalian species and, at least in the livers of sheep and calves, the total content of these thionein-bound cations seems to depend upon the zinc-status of the animal (Stonard and Webb, 1976).

It has been suggested that the intestinal copper- and zinc-thioneins function in the transport and/or absorption of Cu and Zn, the competition between Cd and these cations for the cation-binding-SH groups of thionein being responsible for the antagonism by the former or the absorption of the latter (Evans et al., 1970). Thus, in addition to any changes in organ distribution, the uptake of Cu and Zn also may be inhibited through the formation of cadmium-thionein in the intestinal mucosa (Stonard and Webb, 1976).

Although inhibition by Cd of iron metabolism probably is due primarily to direct competition between these cations for a mucosal binding site other than thionein (Hamilton and

Valberg, 1974), an indirect response, i.e., secondary to the primary interactions between Cd and Cu or Zn, as suggested initially (Hill et al., 1963), also remains possible (Stonard and Webb, 1976).

It is well known that growth depression and anemia are caused by Cd ingestion (Fox et al., 1971; Cousins et al., 1973; Sansi and Pond, 1974; Maji and Yoshida, 1974), and iron deficiency anemia is produced mainly by an inhibition of iron absorption (Freeland and Cousins, 1973; Prigge et al., 1977). To determine whether the anemia is caused by iron deficiency or increased destruction of the erythrocytes, Prigge et al. (1977) examined the different effects of dietary and inhaled Cd on hemoglobin and hematocrit levels and they observed no anemia in rats which inhaled Cd, suggesting that the Cd-induced anemia was due to an iron deficiency.

Suzuki and Yoshida (1978) suggested that the growth retardation induced by dietary Cd 50 μg/g is mainly due to reduced food consumption, and anemia in rats is not directly due to an effect of Cd intake, but primarily to an iron deficiency. Pond et al. (1973) reported that 20 or 30 mg of intramuscularly injected iron on day 1 or 4 of the experiment prevented the anemia in rats caused by the feeding of 100 or 200 μg/g Cd diets, but in these experiments, organ Cd contents were not measured. Banis et al. (1969) found that a supplementation of iron in the diet 400 μg/g could completely alleviate the effect of a 100 μg/g Cd diet. Bunn and Matrone

(1966) found the overall effect of Cd to be a lowering of the liver iron.

The most notable finding, upon examination of a reported case of Cd toxicity in swine, was an extreme anemic condition (Alber, 1963). An extreme microcytic hypochromic anemia was observed in young growing pigs fed a 83 µg/g Cd diet (Osuna et al., 1979). It was found that oral or injected iron offered protection against Cd induced anemia in swine (Pond et al., 1973).

In swine production, with modern feeding and management conditions, Cd toxicity is relatively rare. However, borderline toxicities are possible where animals ingest recycled waste materials, such as urban sewage sludge, in which Cd may be concentrated (Neathery and Miller, 1976). Some types of sludge could be considered as a source of iron for young pigs to correct anemias induced by Fe deficiency (Osuna et al., 1979). Two percent sludge has been found to provide a satisfactory source of vitamin B_{12} for the pig. Toxicity from feeding dried sewage sludge included in a normal swine starter ration may occur from a deficiency of available protein or other essential nutrients, or from the accumulation of hazardous chemical residues (Edds et al., 1978).

Reports on the accumulation and interactions when Cd is fed include chicks (Hill et al., 1963), mice and rats (Bunn and Matrone, 1966; Banis et al., 1969), calves (Powell et al., 1964), hens and goats (Anke et al., 1970), ewes and lambs (Mills and Dalgarno, 1972) and pigs (Pond et al., 1973;

Osuna et al., 1979). The interactions reported in these studies were mainly related to iron, copper and zinc, and these elements were chosen for the present study plus calcium which is one of the factors (Factor IV) essential for coagulation.

Cadmium interferes with the functioning of necessary elements such as zinc in certain enzyme systems (Flick et al., 1971; Friberg et al., 1971). Also, a lethal dose of Cd may inhibit mitochondrial oxidative phosphorylation in rat liver and be directly correlated with death (Southard et al., 1974).

Several cations have been studied for their effect on drug metabolizing enzymes found in the hepatic microsomes of rats. Some elements stimulate (Ca, Mg, Mn, Sr), while other inhibit (Ca, Fe, Zn) drug metabolism (Peters and Fouts, 1970). The stimulating cations also increase NADPH-cytochrome P-450 activity (Fouts and Pohl, 1971).

About 9 - 12 hours following the administration of a single dose of cadmium acetate (2.0 mg/kg, ip) to rats and mice, hexobarbital sleeping time was potentiated (Hadley et al., 1974). The same dose of cadmium acetate administered to male Sprague-Dawley rats 3 days prior to sacrifice produced a significant (50 - 80 percent) inhibition of the metabolism of hexobarbital, p-anitroanisole, aminopyrine and zoxazolamine, but not progesterone. Cytochrome P-450 was also significantly decreased in the hepatic microsomes of these cadmium-injected rats. These investigators also demonstrated in vitro inhibition of these same substrates by adding cadmium acetate

(5×10^{-4} - 5×10^{-7}M) directly to incubated isolated hepatic microsomes of untreated rats (Hadley et al., 1974).

Single doses of cadmium chloride (2.5 and 3.75 mg/kg) equivalent to 1.53 and 2.30 mg of Cd/kg administered to rats by ip injection 6 days prior to sacrifice revealed significant decreases in the activities of aniline hydroxylase (27 - 59 percent) and nitroreductase (57 - 63 percent), depending on the dose and on choice of buffered incubation system used in these assessments. The depression of the microsomal cytochrome P-450 content by Cd ranged from 50 - 56 percent compared to that of the controls (Teare et al., 1977).

Mice exposed to subclinical doses of cadmium chloride (3 µg/g) for ten weeks and inoculated with antigen six weeks after discontinuance of exposure had a remarkable decrease in antibody-forming cells, particularly IgG. These results indicate that the immunosuppression continues and is more pronounced several weeks after exposure to Cd than during administration of the metal (Koller et al., 1975).

Electrophoretic separation of rat plasma revealed that both ^{109}Cd and ^{65}Zn were bound to protein in the following manner: ^{109}Cd was associated predominantly with alpha-globulins, while ^{65}Zn migrated along wtih both alpha and beta-globuin fractions. In addition, 18 percent of both isotopes was also found in the albumin fraction. Both isotopes were excreted mainly via the intestinal tract. Results indicate ^{109}Cd follows metabolic pathways different from those of ^{65}Zn (Shaikh and Lucis, 1972).

Traditionally, clinical tests for determining body cadmium levels in persons exposed to the metal have involved analyses of blood and urine (Cotter, 1958). However, studies of animal or human exposure to Cd suggest that neither blood nor urine is a reliable indicator of total body burden of cadmium (Carlson and Fribert, 1957; Petering et al., 1973). Blood is unsuitable because the sojourn of Cd in this tissue is brief, and its concentration in blood is extremely low (Petering et al., 1973). Urine concentration is not well correlated to tissue concentration of cadmium. After prolonged exposure to Cd or until there is renal damage, urine cadmium concentration tends to be low, regardless of apparent high or low concentrations of Cd in organs (Friberg et al., 1971). Several investigators have suggested that hair may serve as useful indicator of Cd levels in man since hair is known to concentrate this metal (Hammer et al., 1971; Nordberg and Nishiyama, 1972).

After absorption, most cadmium is transported in plasma bound to gammaglobulin (Shaikh and Lucis, 1972). However, some may be bound with hemoglobin or metallothionein in erythrocytes (Carlson and Friberg, 1957).

In summary, general clinical symptoms of cadmium toxicity in mammals include anemia, retarded testicular development or degeneration, enlarged joints, scaly skin, liver and kidney damage, reduced growth and increased mortality (Miller, 1971; Powell et al., 1964).

CHAPTER III

MATERIALS AND METHODS

Thirty-six healthy weaned barrows, mixed breed, averaging 9 kg of body weight, were assigned at random to 6 treatment groups, 6 pigs per group: Group I - negative control; Group II - 0.2 mg of aflatoxin B_1/ kg body weight; Group III - 0.2 mg of warfarin/kg body weight; Group IV - 83 µg/g of cadmium diet (given as cadmium chloride); Group V - 83 µg/g of cadmium diet (given as cadmium chloride) plus 0.2 mg of aflatoxin B_1/kg of body weight; Group VI - 83 µg/g of cadmium diet (given as cadmium chloride) plus 0.2 mg of warfarin/kg of body weight. The experiment was designed to compare two levels of cadmium (0.0 and 83 µg/g diet) in three groups of pigs, i.e., control, aflatoxin B_1 and warfarin (see Figure 1).

Cadmium[a] (Cd) was provided daily through the diets during the 6 weeks of experiment. Aflatoxin B_1[b] and warfarin[c] was given daily per os in a gelatin capsule for five days during the fifth week of the experiment (see Figure 2).

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[a]Cadmium chloride, anhydrous. F.W. 183.31, 99 percent pure. Fisher Scientific Company, Fair Lawn, New Jersey 07410.

[b]Aflatoxin B_1 (cryst) dried in situ from chloroform - A grade, M.W. 312.3. Vials of 10 mg Calbiochem, La Jolla, California.

[c]Crystalline warfarin sodium-Coumadin[R]- Endo Laboratories, Inc., Garden City, New York 11530.

CADMIUM (µg/g)	CONTROL 0.0 mg/kg	AFLATOXIN B_1 0.2 mg/kg	WARFARIN 0.2 mg/kg
0	Group I: 6 barrows	Group II: 6 barrows	Group III: 6 barrows
83	Group IV: 6 barrows	Group V: 6 barrows	Group VI: 6 barrows

Figure 1. Distribution of treatment groups: Group I - negative control; Group II -0.2 mg of aflatoxin B_1/kg body weight; Group III - 0.2 mg of warfarin/kg of body weight; Group IV - 83 µg cadmium/g diet (given as cadmium chloride); Group V - 83 µg cadmium/g diet (given as cadmium chloride) plus 0.2 mg of aflatoxin B_1/kg body weight; Group VI - 83 µg cadmium/g diet (given as cadmium chloride) plus 0.2 mg of warfarin/kg body weight. Two pigs per treatment were slaughtered at the end of the fourth week. The rest of the animals were terminated at the end of the experiment.

	Pre-Trial Week	6 Week Trial Period					
CALENDAR DATES	4/2/79	4/9/79	4/16/79	4/23/79	4/30/79	5/7/79	5/18/79
WEEKS	-1	0	1	2	3	4	6
CRITICAL DATES						0 2 4 6	10
AFLATOXIN DOSAGE						XXXXX	
WARFARIN DOSAGE						+++++	
CADMIUM ADMINISTRATION		----------					
SLAUGHTER TIME						↑	↑
NO. OF PIGS	36	36	36	36	36	23	21
NO. SLAUGHTERED	0	0	0	0	0	12	21
NO. OF DEATHS	0	0	0	0	0	1	2

Figure 2. Schedule of activities. Summary of treatments. Cadmium was provided daily through the diets during the 6 weeks of experiment. Aflatoxin B_1 or warfarin was given orally for five days each at 0.2 mg/kg body weight per day as indicated. Two pigs per treatment were slaughtered at the end of the 4th week (5/4/79). The other animals were terminated (5/18/79). One pigs from group IV died 5/6/79; two more pigs from group VI died, one 5/14/79 and the other 5/15/79.

The pure aflatoxin B_1 was reconstituted at 10 mg/ml of chloroform and then the calculated dose for individual animal was transfered into the gelatin capsule where it was dried *in situ* with a nitrogen stream flow. Evaporating to dryness with the nitrogen prevented the oxidation of the aflatoxin. Warfarin doses were calculated for each animal and placed directly into a gelatin capsule. The capsules, containing either drug or placebo, were protected against light and heat by keeping them in the refrigerator or cold cooler in metal foil wrapped beaker until used.

Pigs were housed by treatment in concrete floored and cement block pens with automatic watering devices and standard pig self-feeders. Floors were washed daily or when necessary to provide sanitary conditions. During the pre-trial week, pigs were allowed to adapt to the new conditions and were divided by body weights into the different treatment groups. Plastic ear tags were used for their identification.

The pigs were fed a basal swine starter ration with an 18 percent crude protein basal diet (Table 1) as the control and as the ration to which $CdCl_2$ was added at an 83 µg Cd/g diet level. Feed and water were provided *ad libitum*.

All rations were tested weekly for aflatoxin B_1[d] and found an average of <20 ng/g for both the control and the Cd ration. Feeds from each of the 6 treatment groups were

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[d]Thin Layer Chromatography. Screening methods for corn, official first action, Method 1, Chapter 26, Association of Official Analytical Chemists, P.O. Box 540, Benjamin Franklin Station, Washington, D.C. 20044.

submitted for pesticide analyses[e] and microbiologic determinations of pathogens[f] on the first and last day of the experiment and were found negative in all the cases.

Feed and water were tested for Ca++, Cd++, Cu++, Fe++ and Zn++ by atomic absorption[g] using standard procedures[h] (see Table 2).

Table 1. Basal diet composition*

Ingredient	Percent
Corn meal	71.80
Soybean meal	25.00
Dyna-fos	1.70
Limestone	0.80
Iodized salt	0.25
Trace mineral mix	0.10
Vitamin premix (UF)	0.10
ASP-250	0.25

*Swine feed starter ration, 18.71 percent protein, medicated, University of Florida.

Table 2. Metal concentration of feed and water (µg/g)

Sample	Group	Ca++	Cd++	Cu++	Fe++	Zn++
Feed	Control	1500.0	< 0.01	16.0	200.0	225.0
	Cd diet	2100.0	88.00	16.0	225.0	225.0
Water	Pen 1*	0.0	< 0.01	< 0.01	0.1	0.13
	Pen 2	0.0	< 0.01	< 0.01	0.1	0.19
	Pen 3	0.0	< 0.01	< 0.01	0.1	0.20
	Pen 4	0.0	< 0.01	< 0.01	0.1	0.20
	Pen 5	0.0	< 0.01	< 0.01	0.1	0.12
	Pen 6	0.0	< 0.01	< 0.01	0.1	0.11

*Pens 1 - 6 are related to treatment groups 1 - 6 respectively.

[e]IFAS, Pesticide Department, University of Florida.

[f]IFAS, Microbiology Department, University of Florida.

[g]Perkin-Elmer atomic absorption unit.

Body weights (BW) were recorded each week for each animal at the same hour. Blood samples were taken weekly except during the fifth week when blood was obtained on days 0, 2, 4, 6 and 10 post-treatment with both aflatoxin B_1 and warfarin. Blood samples were procured by anterior vena cava puncture. Blood samples were collected in tubes[i] with sodium heparin for white blood cell counts (WBC), red blood cell counts (RBC), packed cell volumes (PCV), mean corpuscular volume (MCV) and hemoglobin (Hb); with sodium citrate for prothrombin time (PT) and activated partial thromboplastic time (APTT); with potassium edetate for fibrinogen (F) analyses. Serum samples were obtained for alkaline phosphatase (AP), sorbitol dehydrogenase (SDH), aspartate aminotransferase (SGOT), blood urea nitrogen (BUN), total protein (TP) and protein electrophoresis: Albumin (A), alpha globulin (αG), beta globulin (βG) and gama globulin (γG).

All blood samples drawn were immediately chilled and kept cold in a cooler containing ice bags. The hematologic and enzymatic parameters, including F, were run within 12 hours after bleeding, except PT and APTT, which were run within the first four hours.

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[h] IFAS, Soils Department, University of Florida.

[i] B-D vacutainer, Becton, Dickinson and Co., Rutherford, New Jersey.

Hematologic determinations of RBC, WBC, PCV, MCV and Hb were performed by Coulter Counter model ZBI,[j] Coulter Channelyzer,[j] Coulter RBC/MCV/PCV Computer,[j] Coulter Hemoglobinometer[j] using standard procedures.[j] A fibrometer precision coagulation timer[k] was used for determinations of PT[k] and APTT.[l] Thromboplastic extract[l] and platelet factor 3 reagent (Partial Thromboplastin),[l] 0.025 M calcium chloride[l] were used in the standard procedures for PT and APTT respectively, as well as Verify Normal Citrate[l] and Verify Abnormal Citrate[l] used as controls for samples drawn into citrate. The heat precipitation technique (Millar, Simpson and Stalker, 1971) was the method utilized for the assay of plasma fibrinogen.

Enzymes analyses were performed with a centrifugal analyzer[m] using standard procedures, Calbiochem-Behring reagents[n] and Caltrol II[n] as control serum.

A refractometer was used to determine the serum TP. The A, αG, βG and γG were determined by the standard method of cellulose acetate serum protein electrophoretic technique.[o]

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[j]Coulter Electronics, Inc., Hialeah, Florida.

[k]Baltimore Biological Laboratory, Baltimore, Maryland.

[l]General Diagnostic, Division of Warner-Lambert Co., Morris Plains, New Jersey.

[m]Geamsac Centrifugal Analyzer, Electro Nucleonics, Inc., Fairfield, New Jersey.

[n]Calbiochem-Behring Corp., La Jolla, California.

[o]Gelman Electrophoresis System, Ann Arbor, Michigan 48106.

The materials included a deluxe regulated power supply,[o] sepratek electrophoresis chamber,[o] celulose polyacetate electrophoresis trips (Sepraphore III),[o] sepratek-4 applicator,[o] high resolution buffer,[o] staining solution for serum proteins (Ponceau S capsules),[o] absorbent pads,[o] sepra clear II solution,[o] glass slides[o] and a DCD-16 computing densitometer.[o]

Fecal samples were examined by a standard flotation technique for parasitic ova during the pre-trial, fourth and sixth week of the experiment. Determination of serum freedom of leptospiral titers[p] was performed at the end of the fourth week.

At the end of the fourth week (5/4/79), and prior to the treatment with aflatoxin B_1 and warfarin, two pigs were slaughtered ("first slaughter") from each treatment group in order to collect tissues (kidney, liver and muscle) for histopathology and metal analyses.[h] The rest of the animals were slaughtered ("second slaughter") at the end of the sixth week (5/18/79) for collection of the same tissue samples. The animals were euthanatized (electric shock) and exsanguinated (jugular vein) and samples were obtained: liver, kidney and muscle. Three animals died after the first slaughter; one from group IV (5/4/79) and the other two from group VI (5/14/79 and 5/15/79); these animals were treated in the same way as those at the necropsy.

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[p]College of Veterinary Medicine, Department of Preventive Medicine, University of Florida.

Bladder urine was also taken from each animal during the necropsy with sterile syringe and sterile needles (18 x 1 1/2) for analyses of protein, glucose, ketones, specific gravity and pH, using colorimetric dip strips[q] and a refractometer. Urines were also submitted for metal analyses.[h]

Statistical analyses were performed by application of an analyses of variance, ANOVA, to the data. Testing for significant differences on any particular day between groups was done by "F" test analyses.[r]

The purpose of this experiment was to compare the toxic effects of aflatoxin B_1, a dihydrofuranocoumarin, and warfarin, a 3-(α-acetonyl-benzyl)-4 hydroxycoumarin; and also to determine whether an additive effect from either aflatoxin B_1 or warfarin occurs when Cd is present in the diets of young pigs.

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[q]Ames Company, Division Miles Laboratories, Inc., Elkhart, Indiana 46514.

[r]IFAS Statistics Department, University of Florida, Gainesville, Florida.

CHAPTER IV

RESULTS

Following the design of the experiment, the data results will be presented in two ways. First, the pigs in pen I, II and III were considered as one 0 Cd control group and animals in pen IV, V and VI as one 83 µg/g Cd diet treated group. This included the first four weeks of the experiment and prior to dosing with aflatoxin B_1 or warfarin. Second, the pigs in each pen were considered separately for a total of 6 treatment groups, including negative and positive cadmium controls, and for dosing with either aflatoxin B_1 or warfarin, while exposed to the two levels of Cd for an additional 10 days (Figures 1 and 2).

Performance

Depressed rate of growth (Figure 3 and Table 3) and feed consumption (Table 3) were evident in the pigs consuming 83 µg/g Cd in the diet. The control pigs gained 0.123 kg more per pig per day on the average than those consuming the Cd diet through the fourth week. Feed consumption averaged 0.10 kg per pig per day less for those receiving the Cd diet. A decrease in feed efficiency was also noted, since those receiving the Cd diet required 0.34 kg of feed more per kg of grain than the controls.

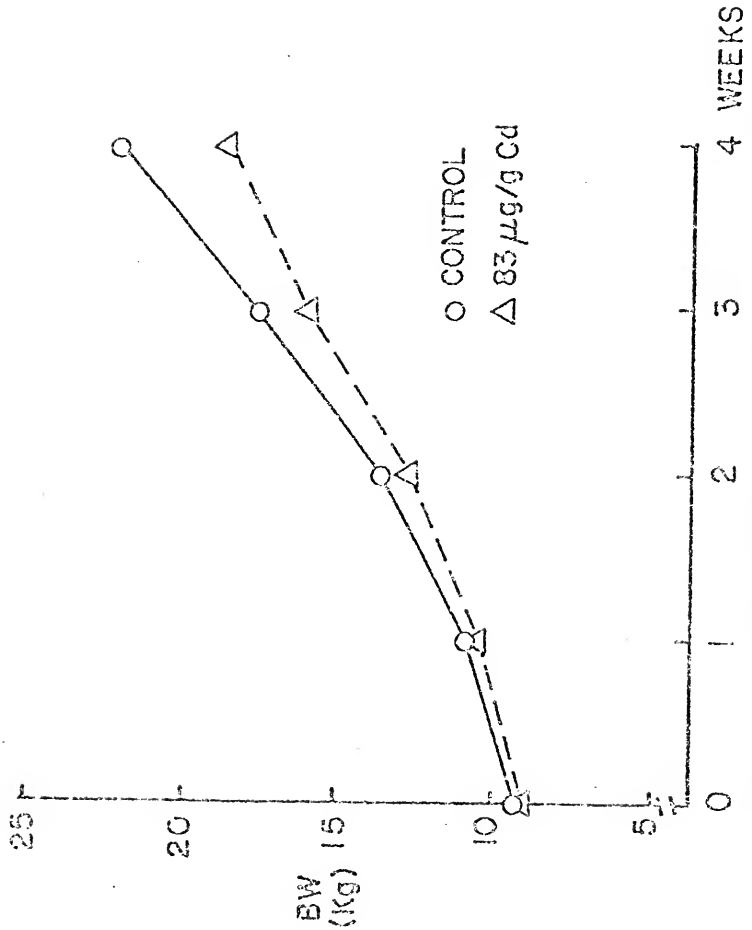

Figure 3. Mean weekly body weights (BW) in young pigs exposed to control or 83 ug/g Cd diets.

Table 3. Body weight and feed consumption during the first 4 weeks of the experiment.

ITEM	CADMIUM INTAKE	µg/g diet
	0	83
Number of pigs per group	18	18
Average feed consumed, kg/pig/day	0.827	0.728
Body weight		
Average initial, kg	9.07	9.02
Average final, kg	22.23	18.73
Average daily gain, kg	0.470	0.347

After 5 daily dosings with aflatoxin B_1, the animals in group II showed a significant weight loss (Figure 4 and Table 4) and those in group V showed decreased feed consumption (Table 4). Pigs in group II (aflatoxin B_1 group) were losing 0.34 kg of BW per animal per day, and those in group V (Cd + aflatoxin B_1 group) were losing 0.05 kg of BW per animal per day. Feed consumption (Table 4) of groups II and V averaged 0.825 and 0.498 kg respectively per pig per day less than those in group I (control group).

After 5 daily dosings with warfarin, the animals in groups III and VI had depressed growth (Figure 4 and Table 4) and feed consumption (Table 4) as compared to those in the control group. Pigs in group I (control group) gained 0.01 kg more per pig per day on the average than those in group III (warfarin group) and gained 0.41 kg more per pig per day on the average than those in group VI (Cd + warfarin group). Feed consumption per pig of groups III and VI averaged 0.05 and 0.338 kg respectively per day less than group I (control group).

During the 10 days after initiation of the oral dosing with aflatoxin B_1 or warfarin, the pigs in group I (control group) gained 0.078 kg more per pig per day on the average than those consuming Cd in group IV (Figure 4 and Table 4). Feed consumption during this time averaged 0.108 kg per pig per day less for pigs in group IV (Cd group).

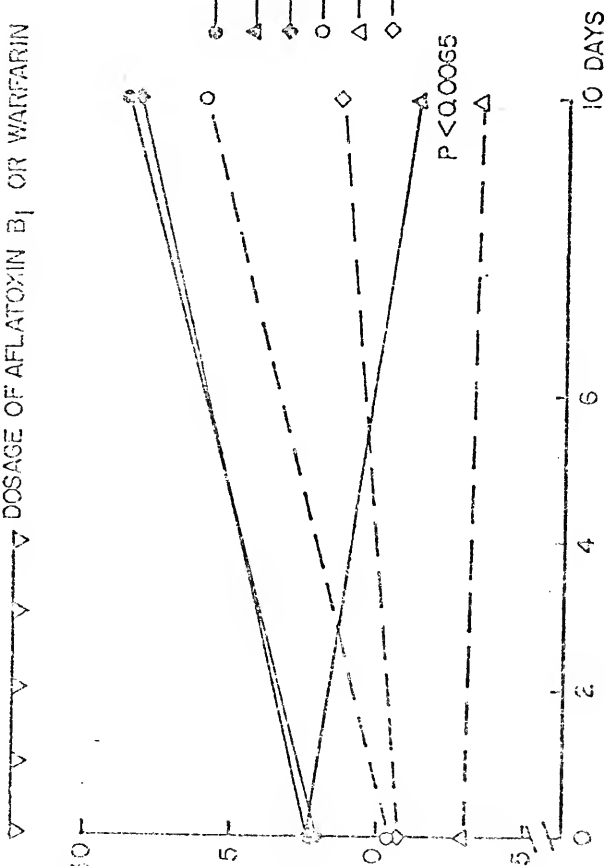

Figure 4. Mean body weights (BW) in young pigs exposed to control or 83 µg/g Cd diets and treated with five daily oral doses of 0.2 mg/kg of aflatoxin B_1 or warfarin as indicated.

Table 4. Body weight and feed consumption during the 10 days after initiation of the oral administration of aflatoxin B_1 or warfarin.

ITEM	TREATMENT GROUPS					
	I	II	III	IV	V	VI
Number of pigs per group	4	4	4	3	4	4
Average feed consumed, kg/pig/day	0.988	0.163	0.938	0.880	0.490	0.650
Body weight						
Average initial, kg	22.1	22.3	22.3	19.7	17.2	19.4
Average final, kg	28.4	18.9	28.5	25.3	16.7	21.5
Average daily gain, kg	0.63	-0.34	0.62	0.56	-0.05	0.22

Hematology

The MCV, Hb and PCV (Figures 5, 7 and 9) of the 83 µg/g Cd diet treated group declined steadily from days 7 through 37. The MCV and Hb of the Cd pigs were significantly lower than those in the control group at the second and third week ($P < 0.0007$ and $P < 0.0001$ respectively), whereas the PCV values of the same group were significantly lower at the third week ($P < 0.0001$). The RBC (Figure 11) of the pigs on the Cd diet were lower than those in the control group after the third week. An extreme microcytic, hypochromic anemia was observed in the Cd diet group by the fourth week of the experiment (Figures 5, 7 and 9) ($P < 0.0001$).

The mean WBC counts (Figure 13) of the 83 µg/g Cd diet treated group were significantly lower than those of the control group through the fourth week ($P < 0.0056$). One Cd diet pig from pen IV died with pneumonia and anemia at the 27th day of the experiment.

The MCV, Hb, PCV, RBC and WBC changes following the oral administration of aflatoxin B_1 or warfarin are presented in figures 6, 8, 10, 12 and 14 respectively. Pigs receiving aflatoxin B_1 (group II) showed a significant increase in Hb ($P < 0.0188$), PCV ($P < 0.0104$) and RBC ($P < 0.0563$) at the 6th day of the treatment. After the peak was reached, all of these values dropped abruptly to lower levels than those of the control group (group I) by the 10th day of the critical phase of the treatment.

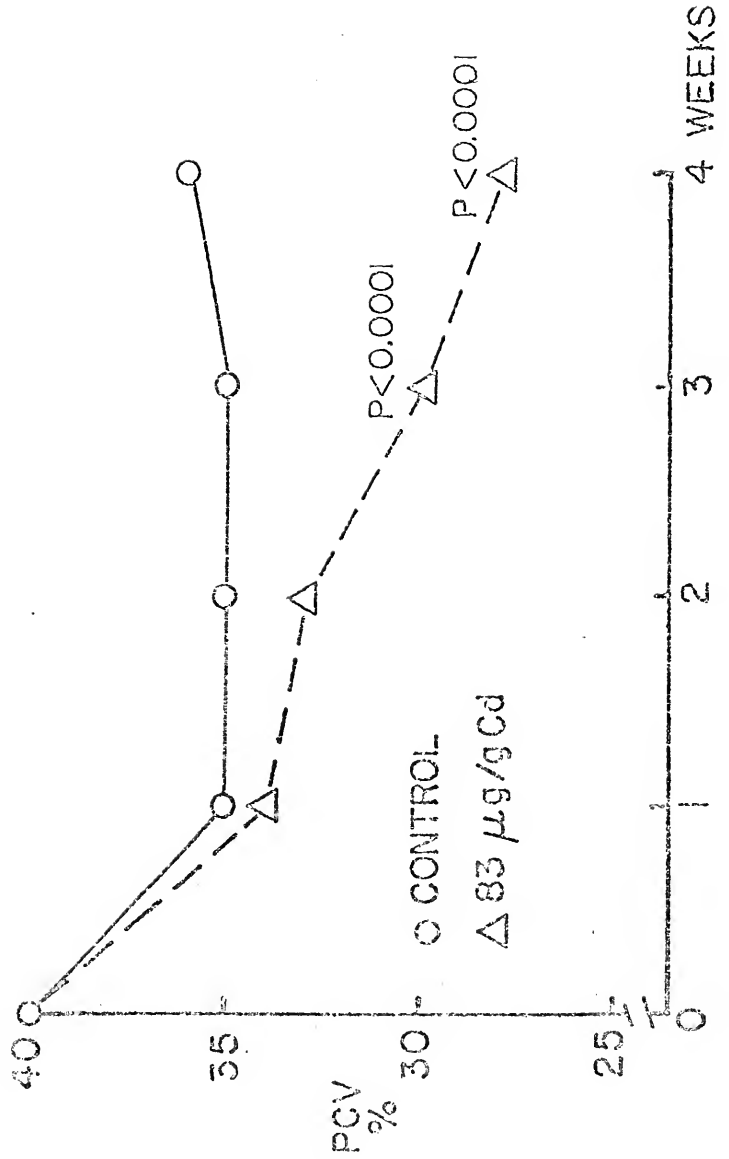

Figure 5. Mean corpuscular volumes (MCV) in young pigs exposed to control of 83 µg/g Cd diets.

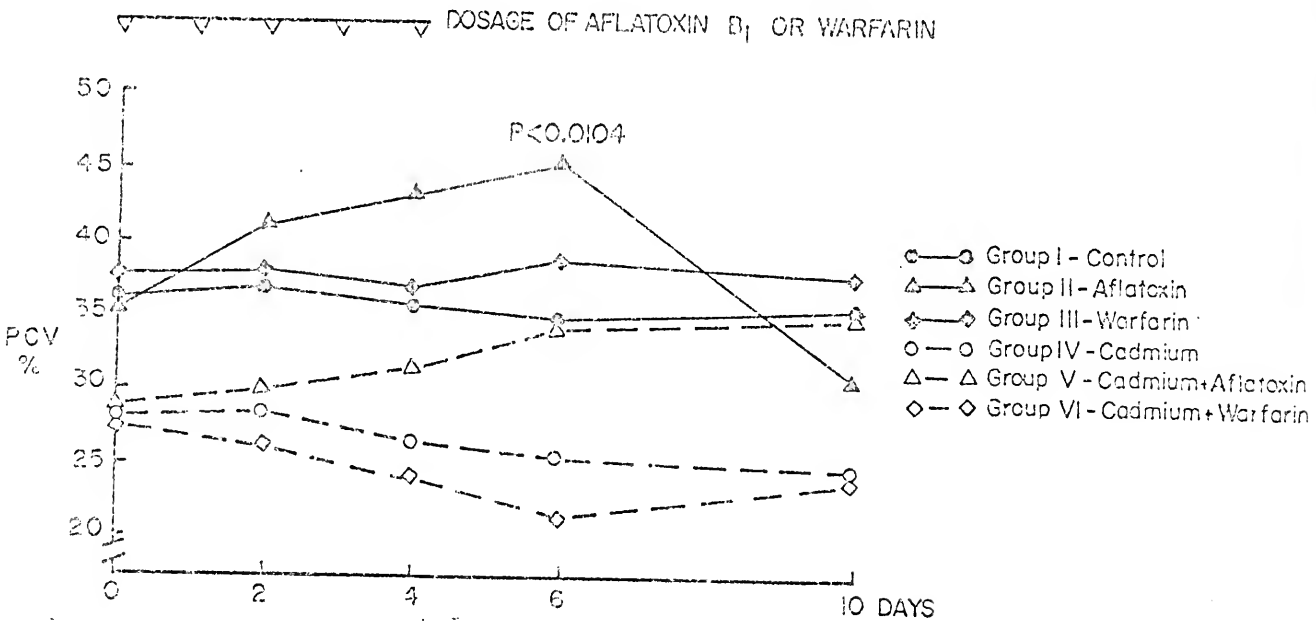

Figure 6. Mean corpuscular volumes (MCV) in young pigs exposed to control or 83 µg/g Cd diets and treated with five daily oral doses of 0.2 mg/kg of aflatoxin B_1 or warfarin as indicated.

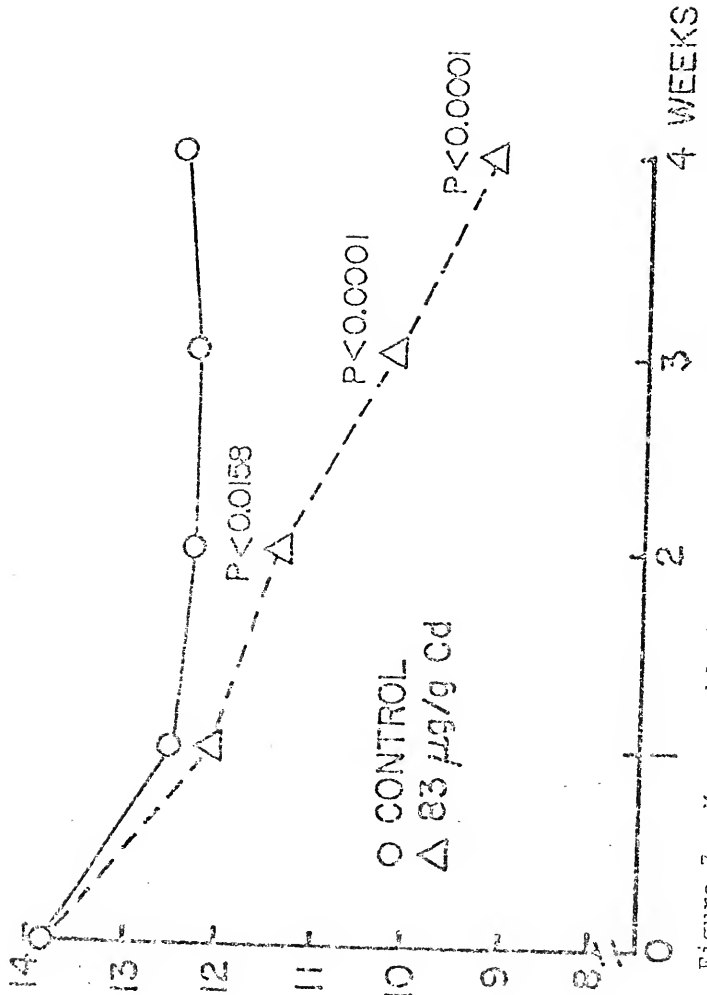

Figure 7. Mean weekly hemoglobin (Hb) concentrations in young pigs exposed to control or 83 ug/g Cd diets.

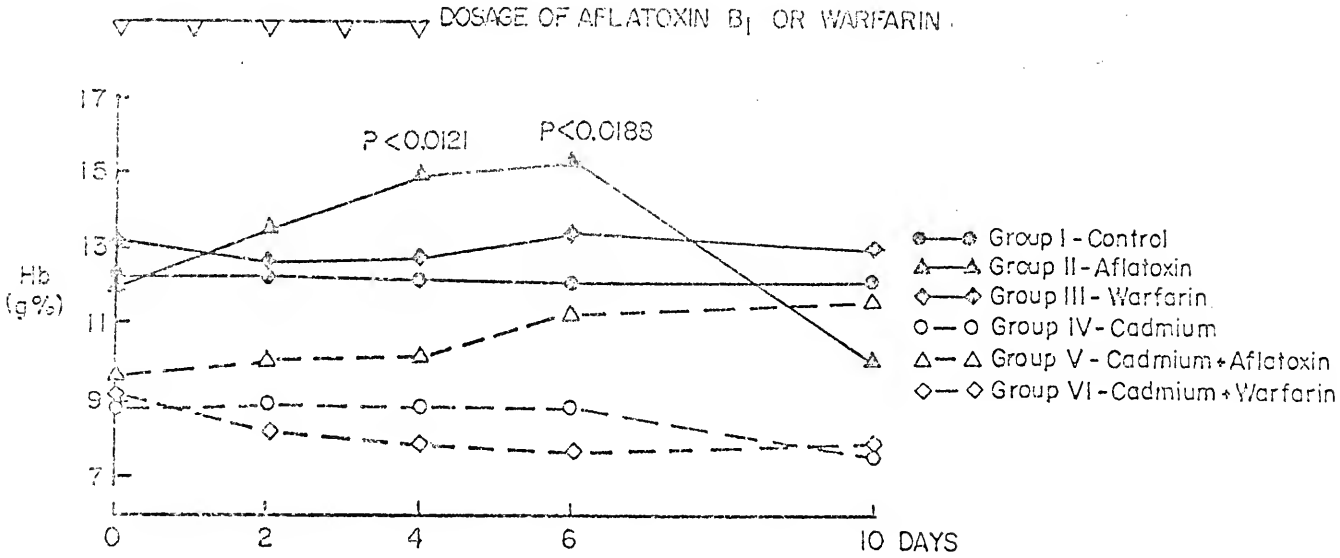

Figure 8. Mean hemoglobin (Hb) concentrations in young pigs exposed to control or 83 µg/g Cd diets and treated with five daily oral doses of 0.2 mg/kg of aflatoxin B_1 or warfarin as indicated.

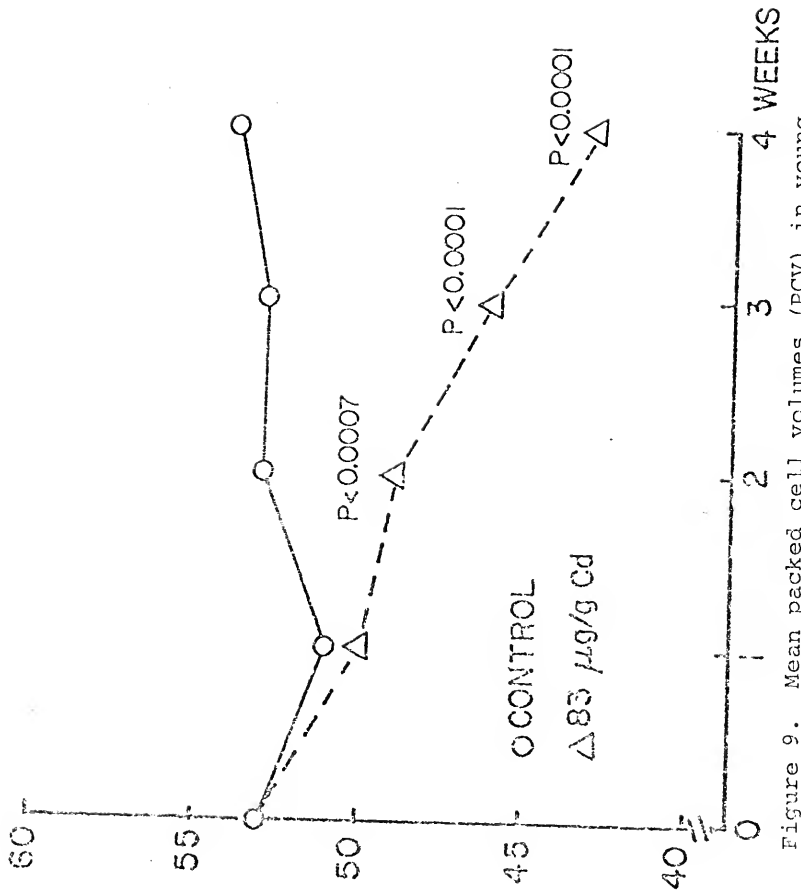

Figure 9. Mean packed cell volumes (PCV) in young pigs exposed to control or 83 μg/g Cd diets.

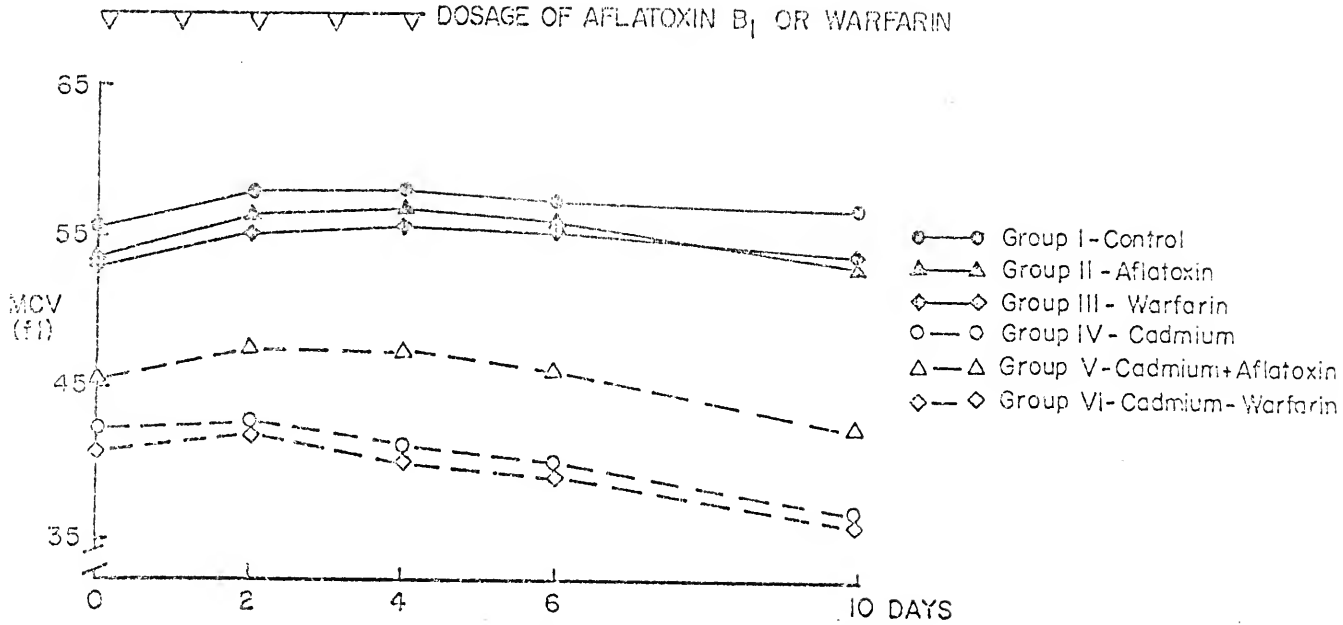

Figure 10. Mean packed cell volumes (PCV) in young pigs exposed to control or 83 ug/g Cd diets and treated with five daily doses of 0.2 mg/kg of aflatoxin B_1 or warfarin as indicated.

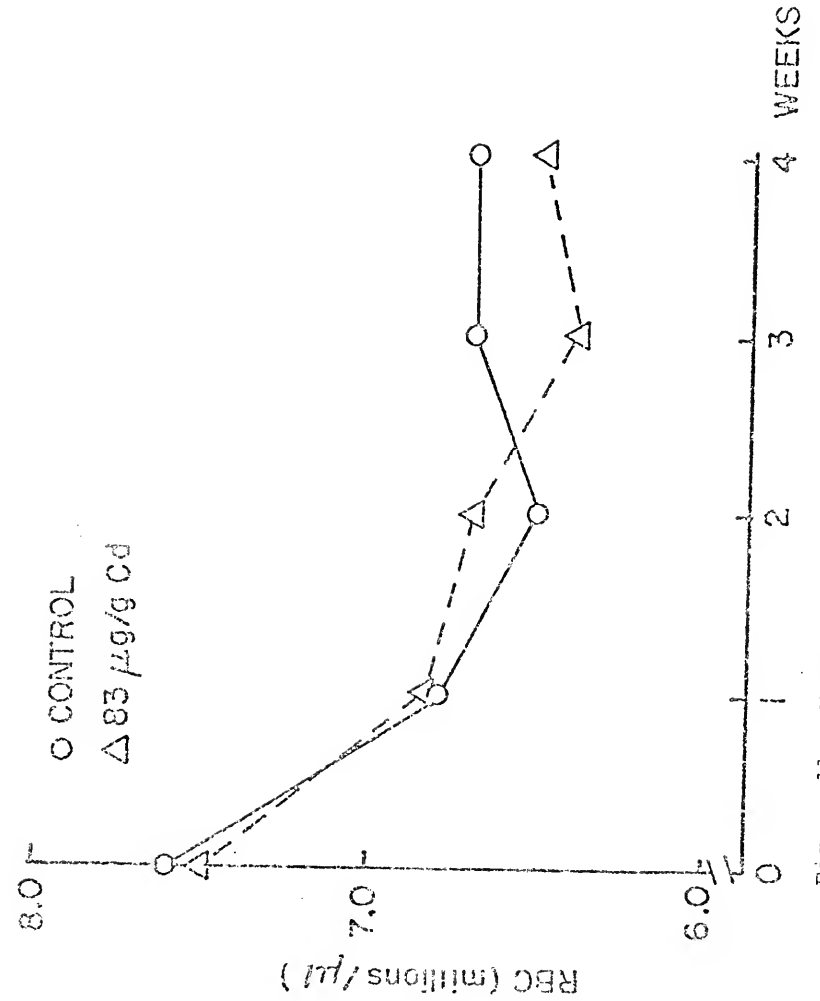

Figure 11. Mean red blood cells (RBC) counts in young pigs exposed to control or 83 μg/g Cd diets.

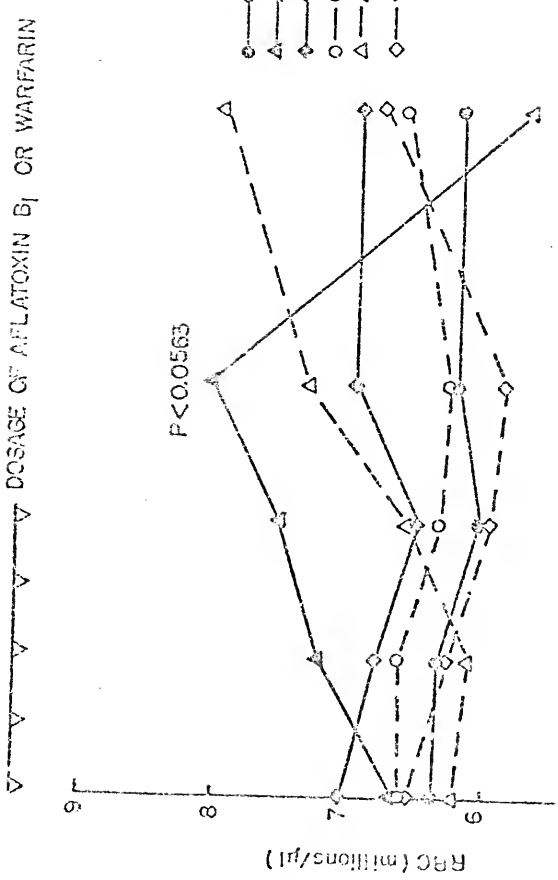

Figure 12. Mean red blood cell (RBC) counts in young pigs exposed to control or 83 µg/g Cd diets and treated with five daily oral doses of 0.2 mg/kg of aflatoxin B_1 or warfarin as indicated.

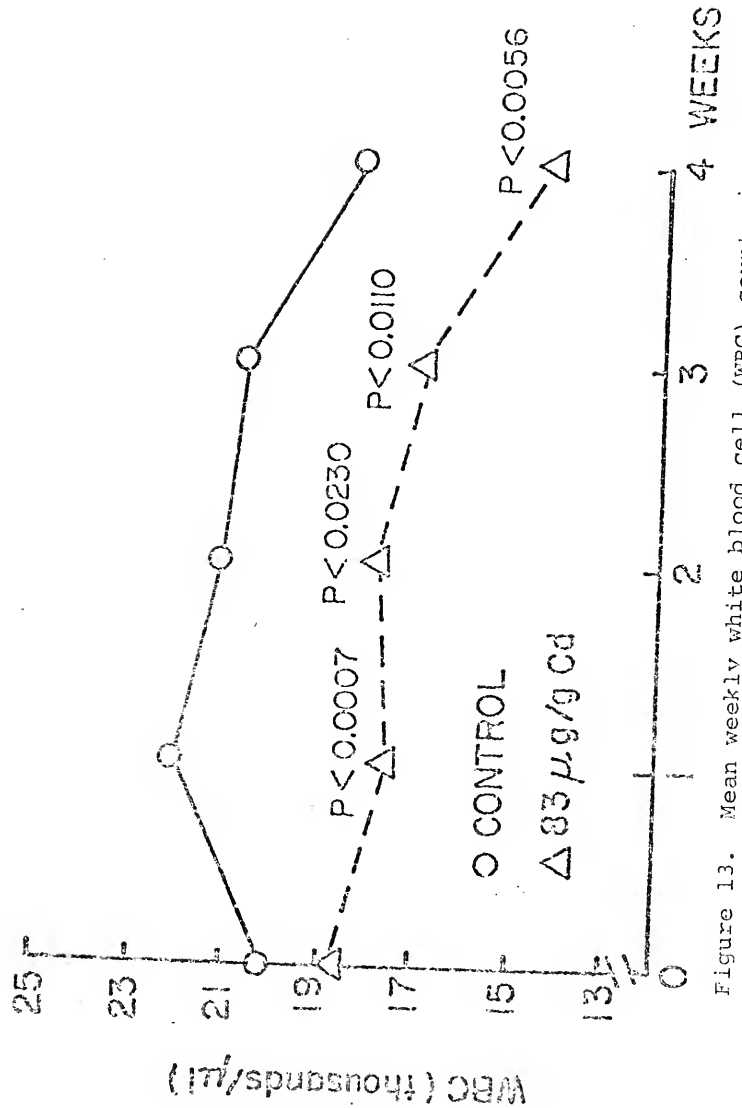

Figure 13. Mean weekly white blood cell (WBC) counts in young pigs exposed to control or 83 μg/g Cd diets.

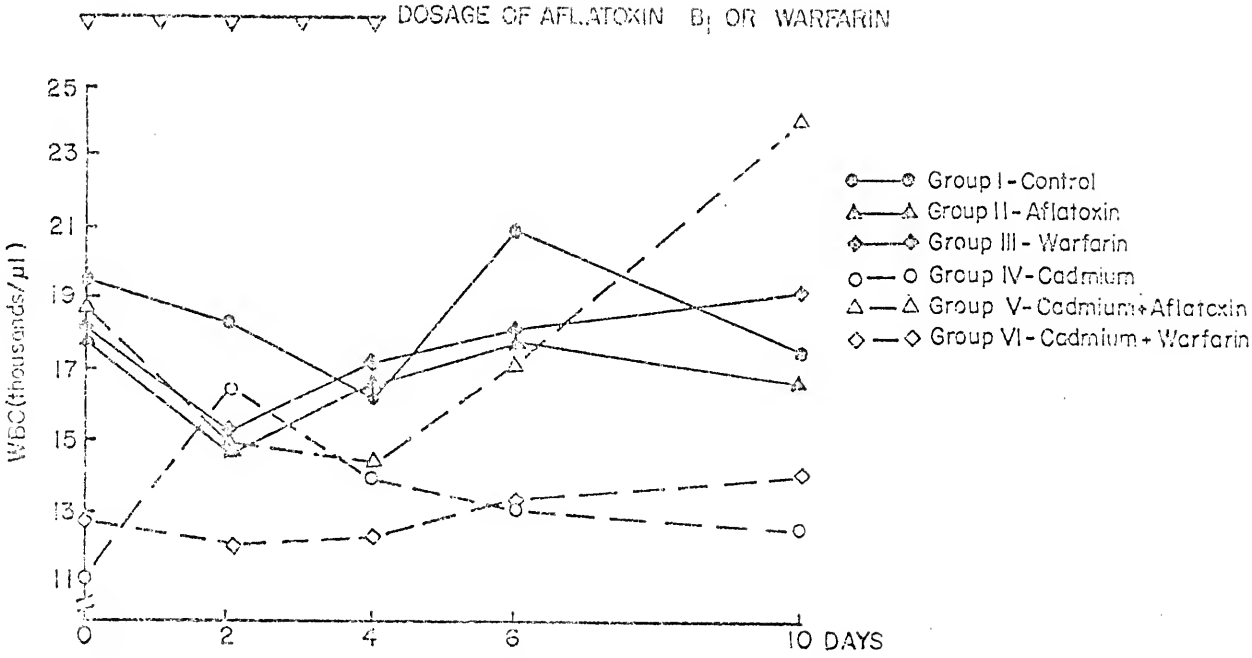

Figure 14. Mean white blood cell (WBC) counts in young pigs exposed to control or 83 µg/g Cd diets and treated with five daily oral doses of 0.2 mg/kg of aflatoxin B_1 or warfarin as indicated.

Pigs treated with Cd diet and aflatoxin B_1 (group V) presented the same tendency to increase the Hb, PCV and RBC levels immediately after the oral dosages at the 4th week. However, the anemic condition of the pigs induced by the Cd diet did not allow these values to reach a significant level.

No significant changes were observed in the MCV or WBC following dosage with either aflatoxin B_1 or warfarin.

When the control values of blood in figures 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 are compared, the following hematologic ranges can be reported for young swine:

1. MCV 54.91 ± 2.19 cubic microns
2. Hb 12.43 ± 1.03 g percent
3. PCV 36.16 ± 2.89 percent
4. RBC 6.56 ± 0.65 millions/µl
5. WBC 19.58 ± 4.27 thousands/µl

Serum Enzymes

Alkaline phosphatase (AP): The levels of AP in the control or 0 Cd diet group increased with time from the beginning through the 4th week (Figure 15). On the contrary, the levels of AP in the 83 µg/g Cd diet treated group decreased (Figure 15) from the beginning to the 4th week of the experiment.

During the 10 days after initiation of the oral dosing with aflatoxin B_1, the pigs in group II (aflatoxin B_1 group) showed a marked and a significant increase in AP levels (Figure 16). This level reached a peak that remained high from the 4th day after aflatoxin B_1 exposure ($P < 0.016$). The AP

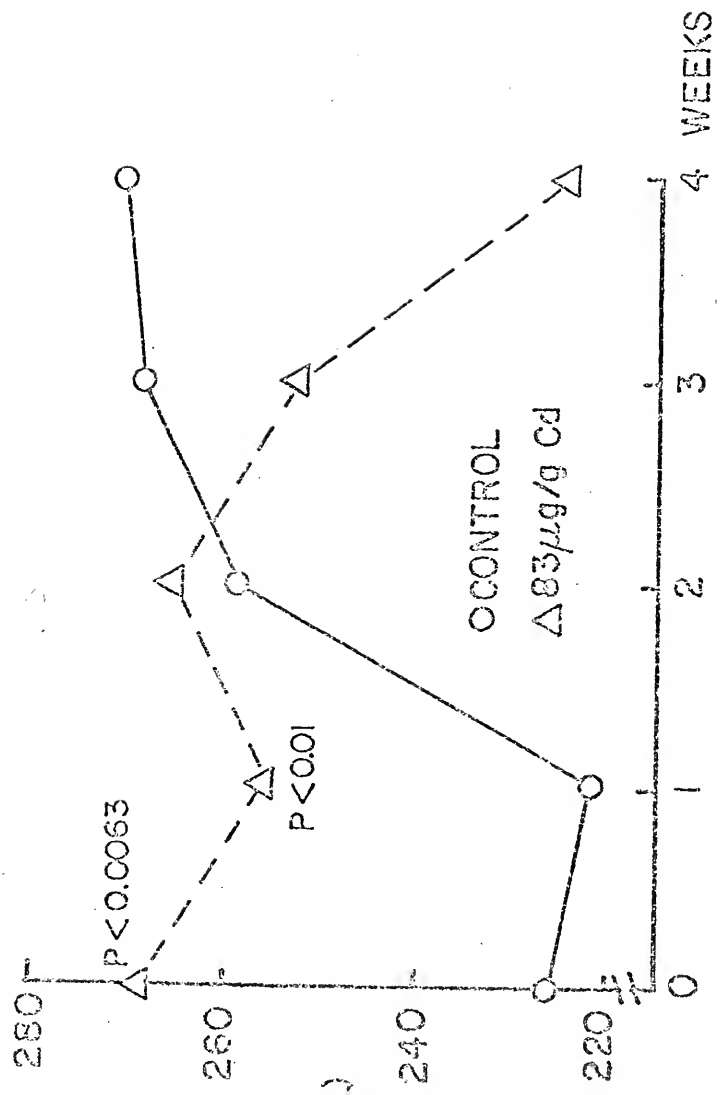

Figure 15. Mean weekly alkaline phosphatase (AP) concentrations in young pigs exposed to control or 83 μg/g Cd diets.

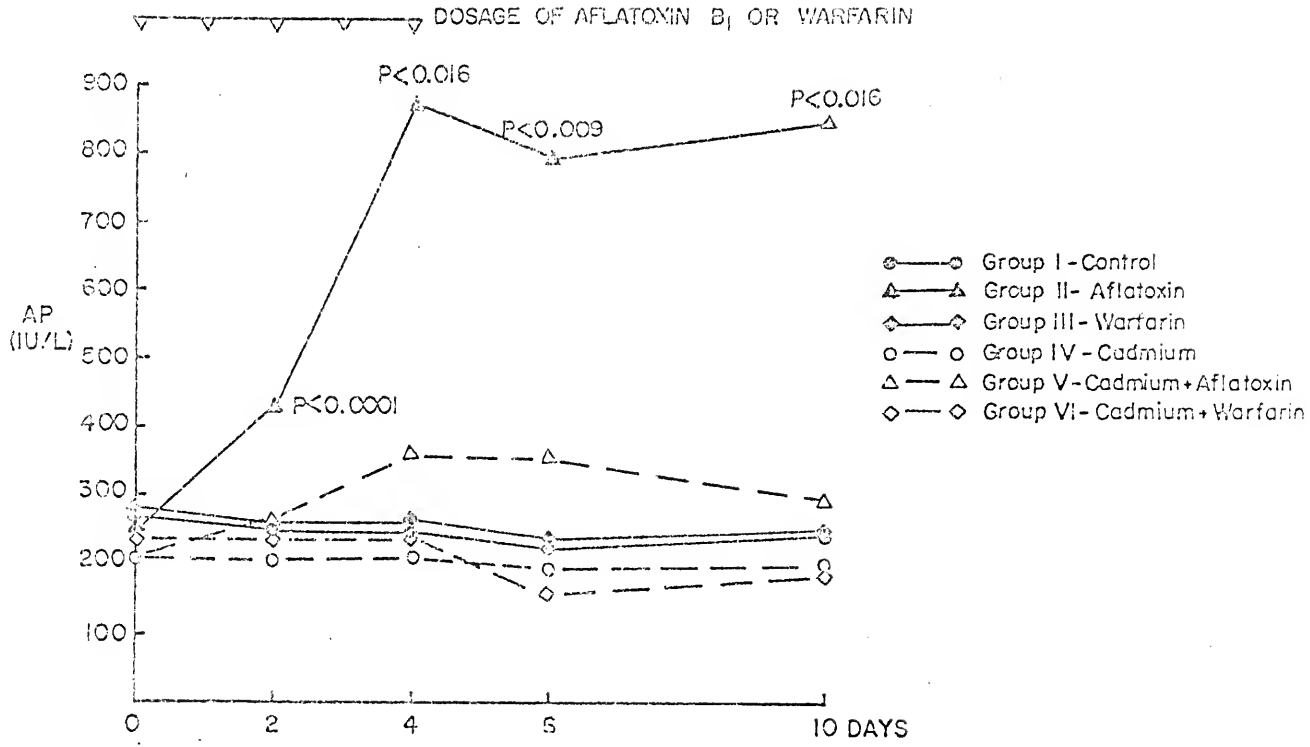

Figure 16. Mean alkaline phosphatase (AP) concentrations in young pigs exposed to control or 83 µg/g Cd diets and treated with five daily oral doses of 0.2 mg/kg of aflatoxin B_1 or warfarin as indicated.

levels in the pigs of group V (Cd + aflatoxin B_1 group) increased moderately after dosing the pigs with aflatoxin B_1 (Figure 16) but not as high as those in group II.

No significant changes in the AP levels were observed during the 10 days after initiation of the oral administration of warfarin (Figure 16) in groups III (warfarin group) and VI (Cd + warfarin group). In the same way, no significant changes were determined in the AP levels in group I (control group) and IV (Cd group) during this period. The AP levels of group IV were lower than those of group I during this 10 day period.

Sorbitol dehydrogenase (SDH): SDH levels (Figure 17) of the 83 µg/g Cd diet treated group were always higher than those from the control group from the beginning ($P < 0.0063$) of the experiment through the 4th week. The SDH levels were significantly higher at the second week ($P < 0.0001$) of the experiment.

During the 10 days after initiation of the oral dosing with aflatoxin B_1 (Figure 18), the pigs in groups II (aflatoxin B_1 group) and V (Cd + aflatoxin B_1 group) showed a significant increase ($P < 0.003$) in SDH levels over those in control group I. The SDH levels in pigs in group II were higher than those in group V at the second day (48 hours) of the treatment. Both levels in these two groups returned to normal by the 10th day of the treatment period.

No significant changes in the SDH levels were observed during the 10 days after initiation of the oral administration

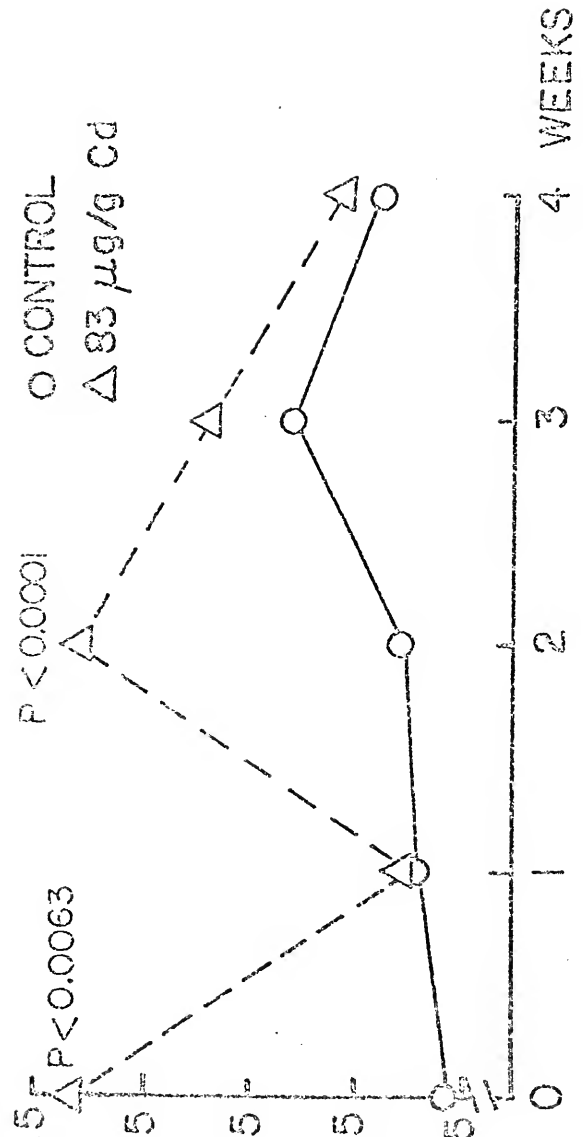

Figure 17. Mean weekly sorbitol dehydrogenase (SDH) concentrations in young pigs exposed to control or 83 µg/g Cd diets.

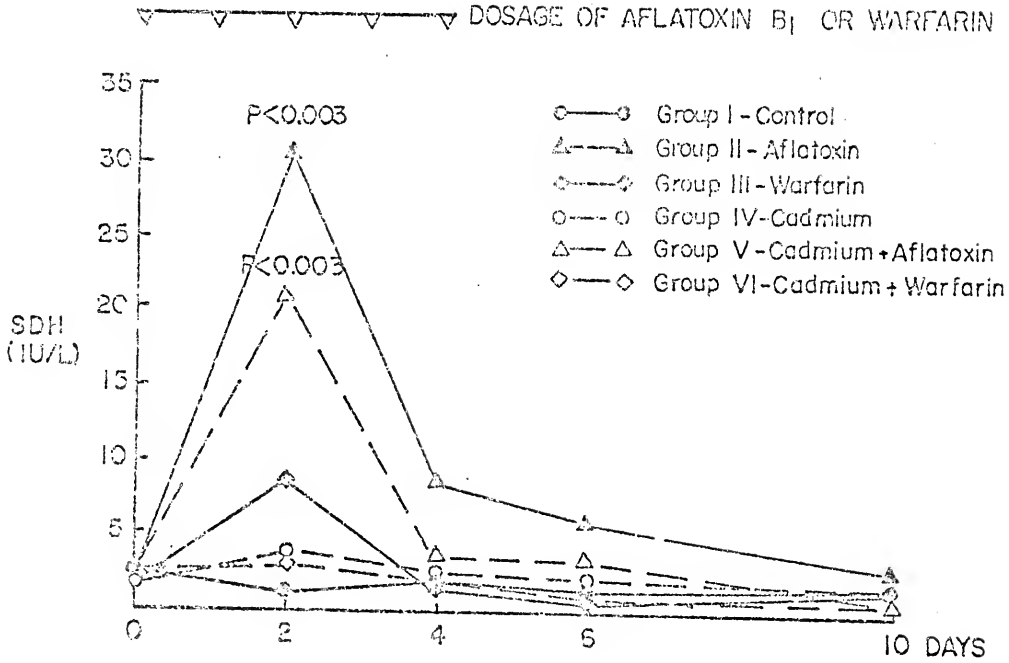

Figure 18. Mean sorbitol dehydrogenase (SDH) concentrations in young pigs exposed to control or 83 µg/g Cd diets and treated with five daily oral doses of 0.2 mg/kg of aflatoxin B_1 or warfarin as indicated.

of warfarin (Figure 18) in groups III (warfarin group) and VI(Cd + warfarin group). Neither did significant changes occur in the SDH levels in group I (control group) nor in group IV (Cd group) during this 10 day period.

Aspartate aminotransferase (SGOT): The levels of SGOT in the control group (Figure 19) decreased with time from the beginning through the 4th week. On the contrary, the levels of SGOT in the 83 µg/g Cd diet treated group showed a tendency to increase (Figure 19) from the beginning up to the 4th week of the experiment. However, there were no significant differences between the SGOT levels of these two Cd diet groups (0 and 83 µg/g) during the first 4 weeks.

During the 10 days after initiation of oral dosing with aflatoxin B_1, the pigs in group II (aflatoxin B_1 group) and V (Cd + aflatoxin B_1 group) showed a significant increase in the SGOT levels (Figure 20). Significant levels in group II reached a peak at the second day (48 hours) ($P < 0.05$) and remained high to the end of the 10th day ($P < 0.03$) of experimental treatment. The SGOT levels in group V were not higher than those of group II ($P < 0.05$) at the second day (48 hours) of treatment.

There was a significant increase ($P < 0.05$) of mean SGOT levels (Figure 20) in group III (warfarin group) by the second day after initiation of the warfarin treatment. The levels returned to normal by the 4th day. No significant changes of SGOT levels were observed in group VI (Cd + warfarin group) during this 10 day period. Neither were significant changes

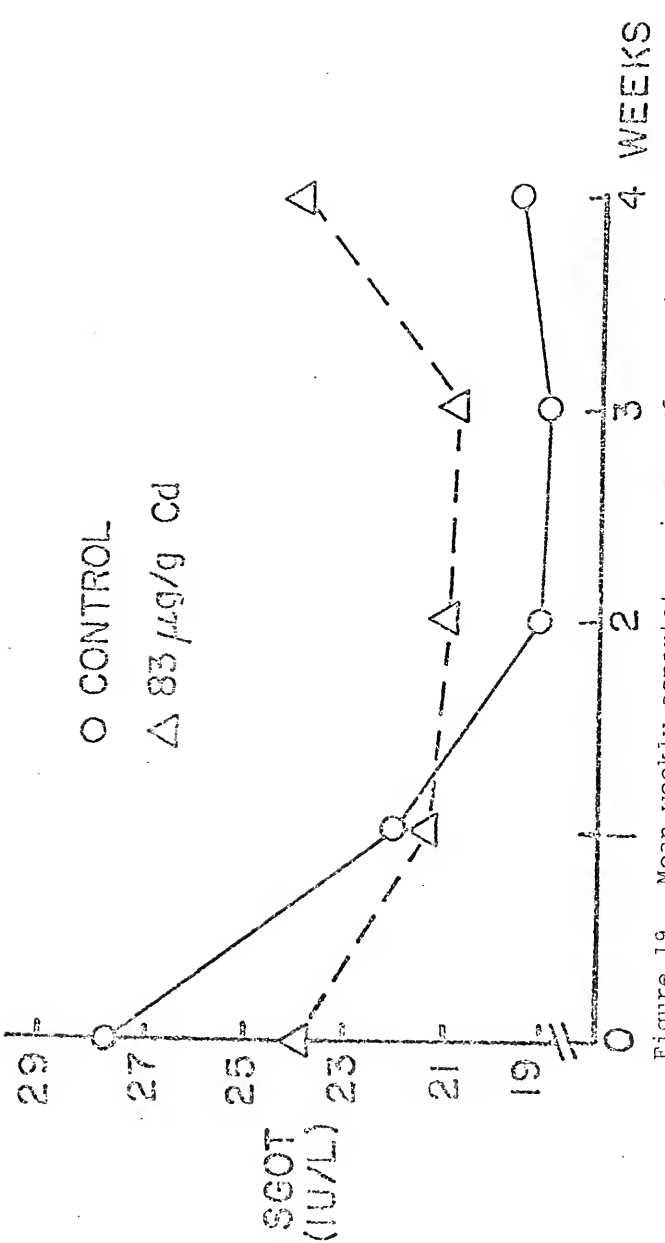

Figure 19. Mean weekly aspartate aminotransferase (SGOT) concentrations in young pigs exposed to control or 83 μg/g Cd diets.

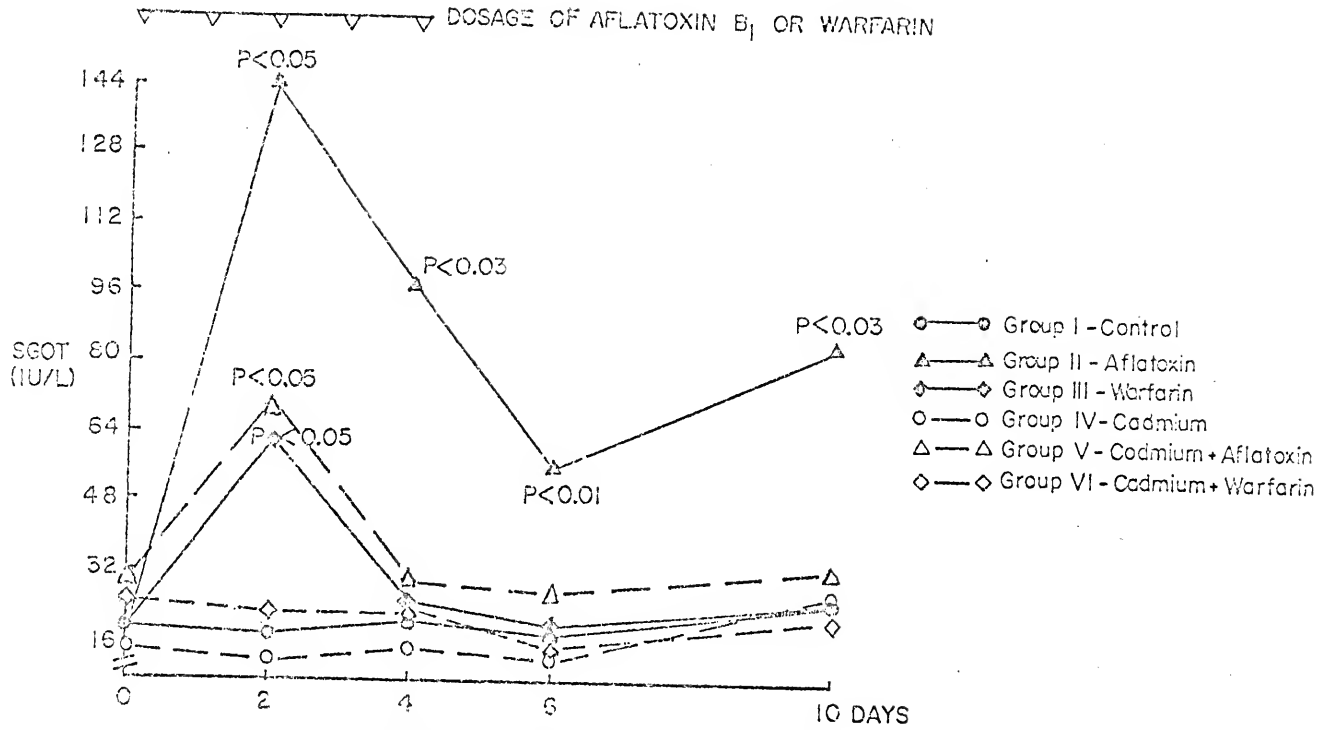

Figure 20. Mean aspartate aminotransferase (SGOT) concentrations in young pigs exposed to control or 83 µg/g Cd diets and treated with five daily oral doses of 0.2 mg/kg of aflatoxin or warfarin as indicated.

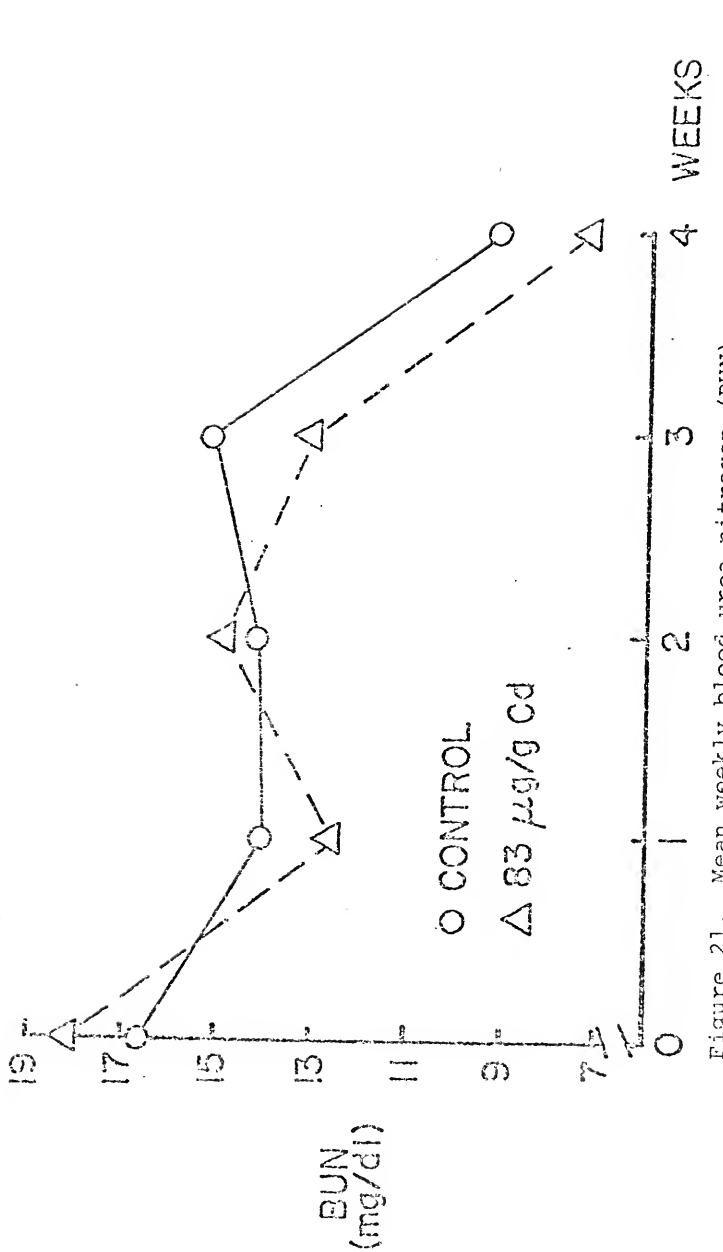

Figure 21. Mean weekly blood urea nitrogen (BUN) concentrations in young pigs exposed to control or 83 μg/g Cd diets.

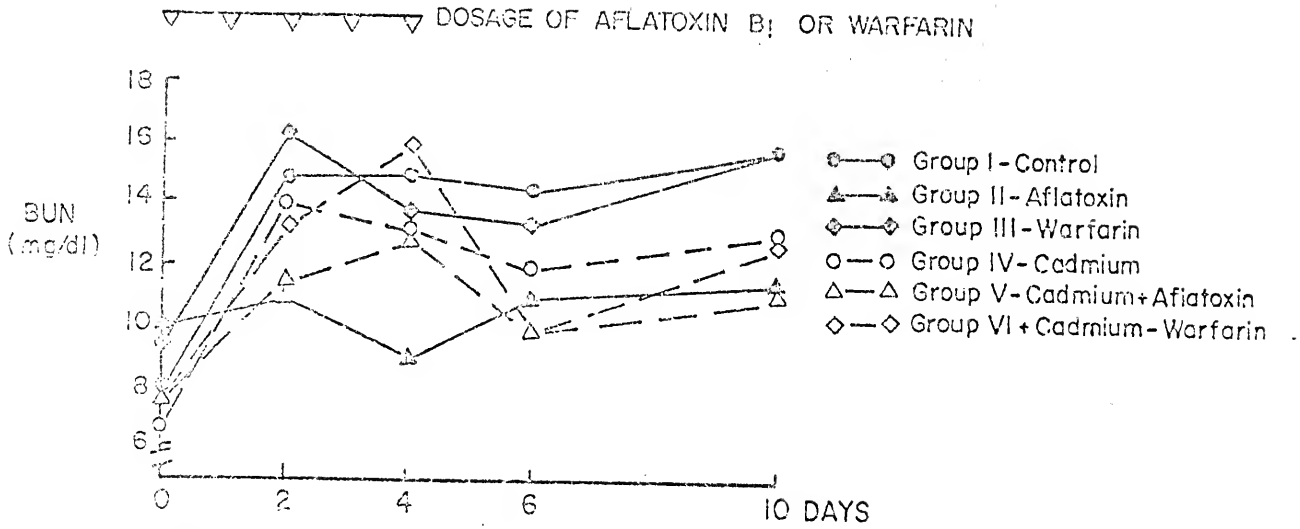

Figure 22. Mean blood urea nitrogen (BUN) concentrations in young pigs exposed to control or 83 µg/g Cd diets and treated with five daily oral doses of 0.2 mg/kg of aflatoxin B_1 or warfarin as indicated.

present in the SGOT levels of group I (control group) nor in group IV (Cd group) during this period.

Blood urea nitrogen (BUN): The mean BUN levels of the 83 µg/g Cd diet treated group of pigs paralleled those values of the control group throughout the trial (Figure 21).

No significant changes or differences develop between groups during the 10 days after initiation of oral administration of aflatoxin B_1 or warfarin (Figure 22).

When the serum enzyme levels of the control groups, presented in Figures 15, 16, 17, 18, 19, 20, 21 and 22 are examined, the following normal ranges can be reported for young swine:

1. AP 226 ± 44.36 IU/dl
2. SDH 2.03 ± 0.97 IU/dl
3. SGOT 21.06 ± 4.27 IU/dl
4. BUN 13.79 ± 2.26 mg/dl

Blood Coagulation

Prothrombin time (PT): There were no significant prolongations in PT between the control and the 83 µg/g Cd diet group (Figure 23) during the first 4 weeks of the experiment. However, during and after the administration of aflatoxin B_1 and warfarin, both groups presented a rapid and marked increase in PT.

During the 10 days during and after oral dosing with aflatoxin B_1, the pigs in group II (aflatoxin B_1 group) showed a significant increase in PT levels (Figure 24) over the group I (control group) at the 4th ($P < 0.0009$), 6th

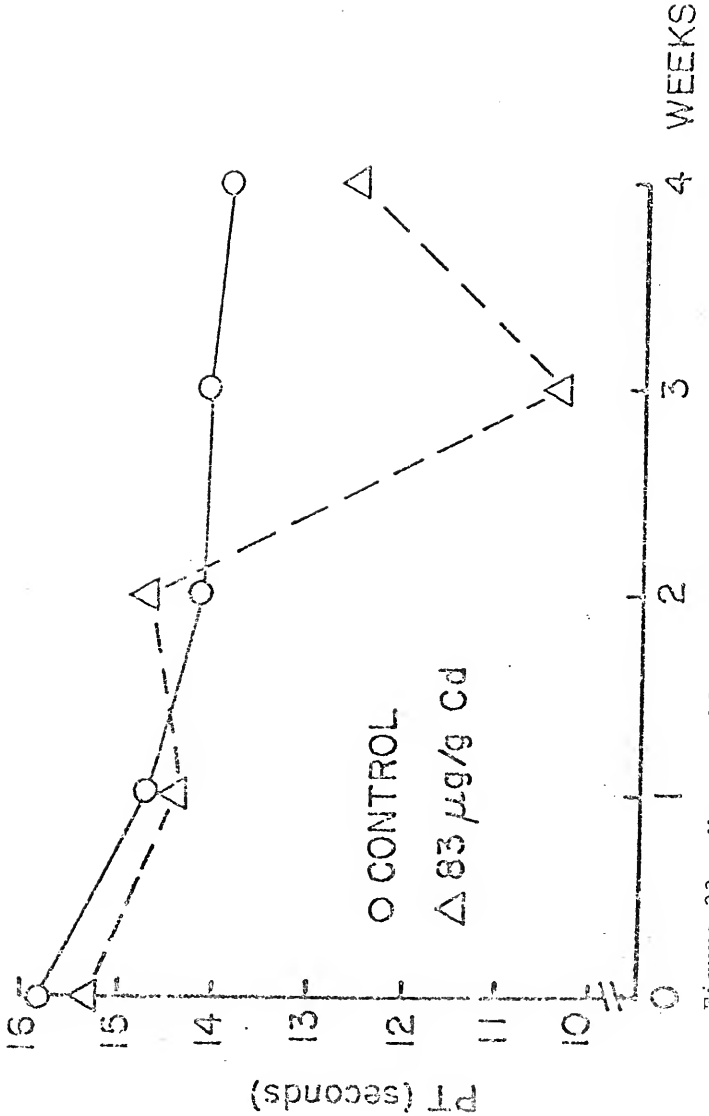

Figure 23. Mean weekly prothrombin times (PT) in young pigs exposed to control or 83 µg/g Cd diets.

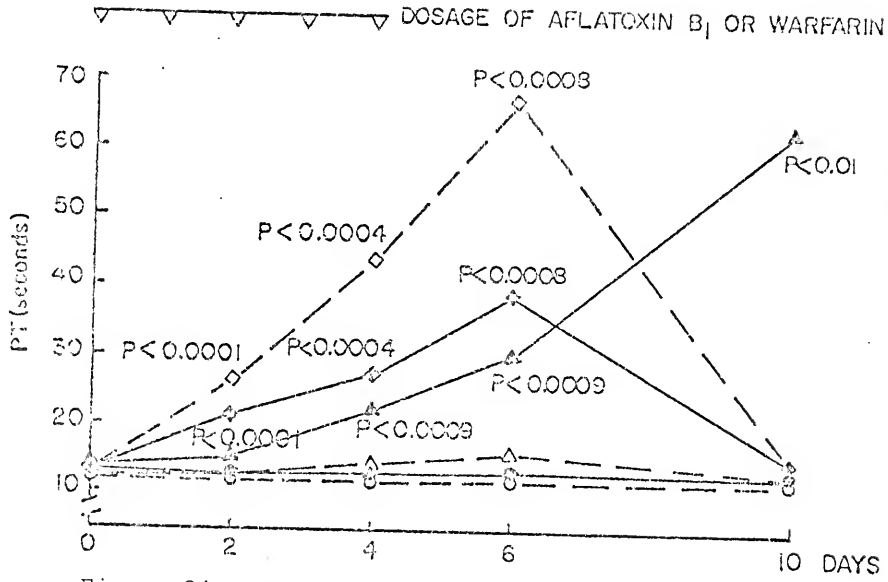

Figure 24. Mean prothrombin times (PT) in young pigs exposed to control or 83 µg/g Cd diets and treated with five daily oral doses of 0.2 mg/kg of aflatoxin B_1 or warfarin as indicated.

($P < 0.0009$) and at the 10th day ($P < 0.01$) of the experimental treatment. The levels of PT in group II were lower than those of group III (warfarin group) and group VI (Cd + warfarin group) during the first 6 days but were more prolonged at the 10th day of the experiment. It was also observed that the maximum peak in PT (10th day) of group II occurred 6 days after the maximum concentration of AP was obtained (Figures 16 and 24). Finally, there were no significant increases in the PT values in group V (Cd + aflatoxin B_1 group) (Figure 24). The levels of group V paralleled the values of group I (control group).

Pigs receiving warfarin showed a more rapid and higher increase in PT than those with aflatoxin B_1 during the first 6 days (Figure 24) but returned to normal at the end of the experiment. The mean PT in group VI (Cd + warfarin group) were higher than those of group III (warfarin group). At the 6th day, both groups III and VI had reached the maximum time ($P < 0.0008$).

No significant changes of PT in groups I (control group) and IV (Cd group) were observed during or after oral administration of aflatoxin B_1 or warfarin. The values of the Cd treated pigs paralleled very closely those of the control group (Figure 24).

Activated partial thromboplastin time (APTT): There were no significant changes in APTT in the control or the 83 µg/g Cd diet treated groups (Figure 25) during the first 4

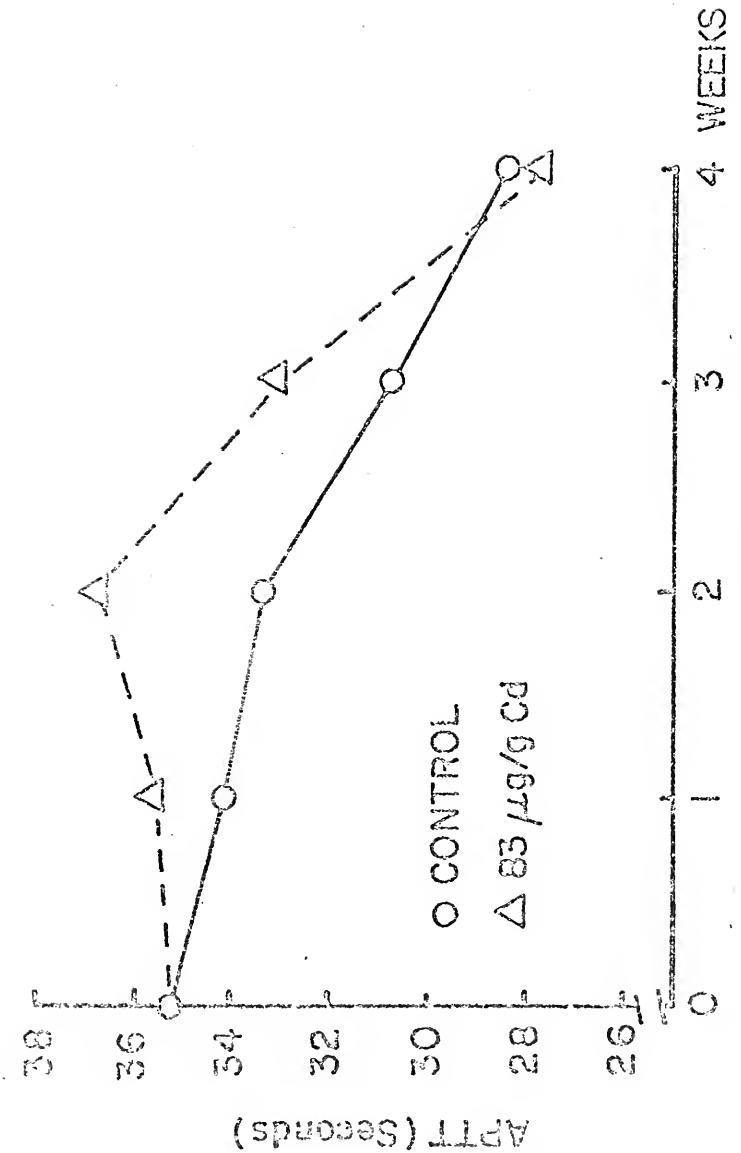

Figure 25. Mean weekly activated partial thromboplastin times (APTT) in young pigs exposed to control or 83 µg/g Cd diets.

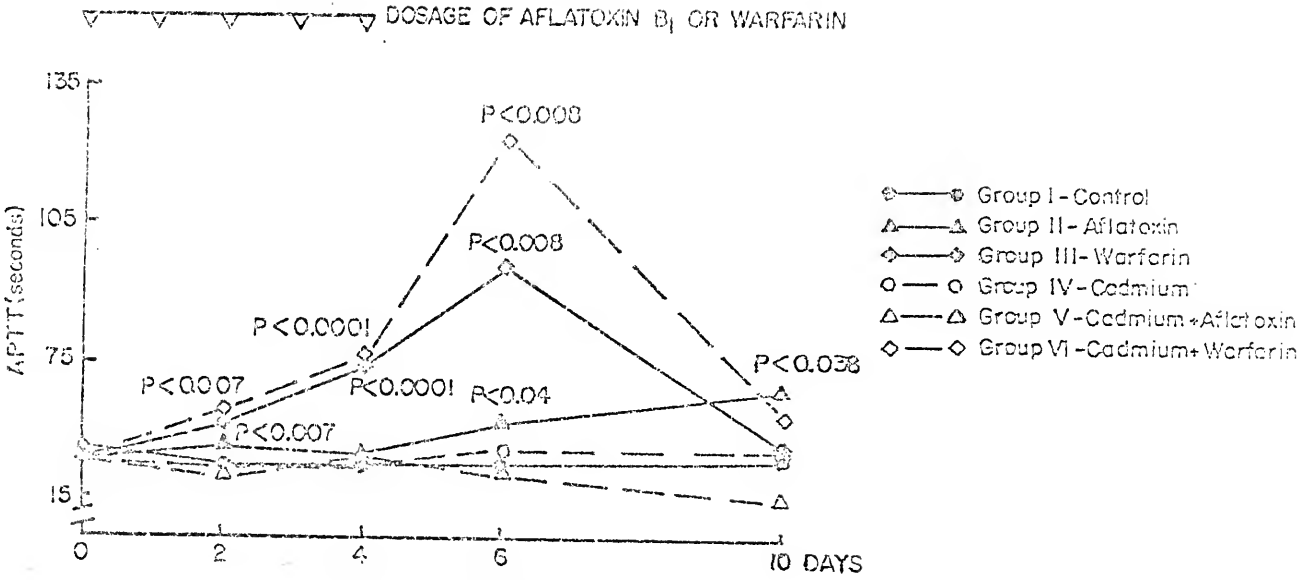

Figure 26. Mean activated partial thromboplastin times (APTT) in young pigs exposed to control or 83 µg/g Cd diets and treated with five daily doses of 0.2 mg/kg of aflatoxin B_1 or warfarin as indicated.

weeks of the experiment. The APTT values in both groups decreased with time through the 4th week.

During the 10 days after initiation of the oral dosing with aflatoxin B_1, the pigs in group II (aflatoxin B_1 group) showed a significantly greater increase in the APTT (Figure 26) over those in group I (control group) at the 6th ($P < 0.04$) and the 10th day ($P < 0.038$) of the experiment. There were no significant increases in the APTT levels in group V (Cd + aflatoxin B_1 group) during and after dosing the pigs with aflatoxin B_1 (Figure 26). The levels of group V paralleled the values of the control group (group I) during the first 6 days and were lower by the end of the experiment.

Pigs receiving warfarin in groups III (warfarin group) and VI (Cd + warfarin group) showed a more rapid and higher increase in APTT than those with aflatoxin B_1 during the first 6 days. The levels returned to near normal after dosing at the 10th day of the experiment. The APTT values in group VI were higher than those of group III and the highest of all treatment groups. At the 6th day, both groups III and VI had reached the maximum ($P < 0.008$).

No significant changes of APTT resulted in groups I (control group) and IV (Cd group) during the 10 days of the experiment. The values of the pigs on the Cd diet closely paralleled those of the control group.

Fibrinogen (F): The F values in the control and Cd pigs treated groups were variable but not significantly different through the experiment (Figure 27).

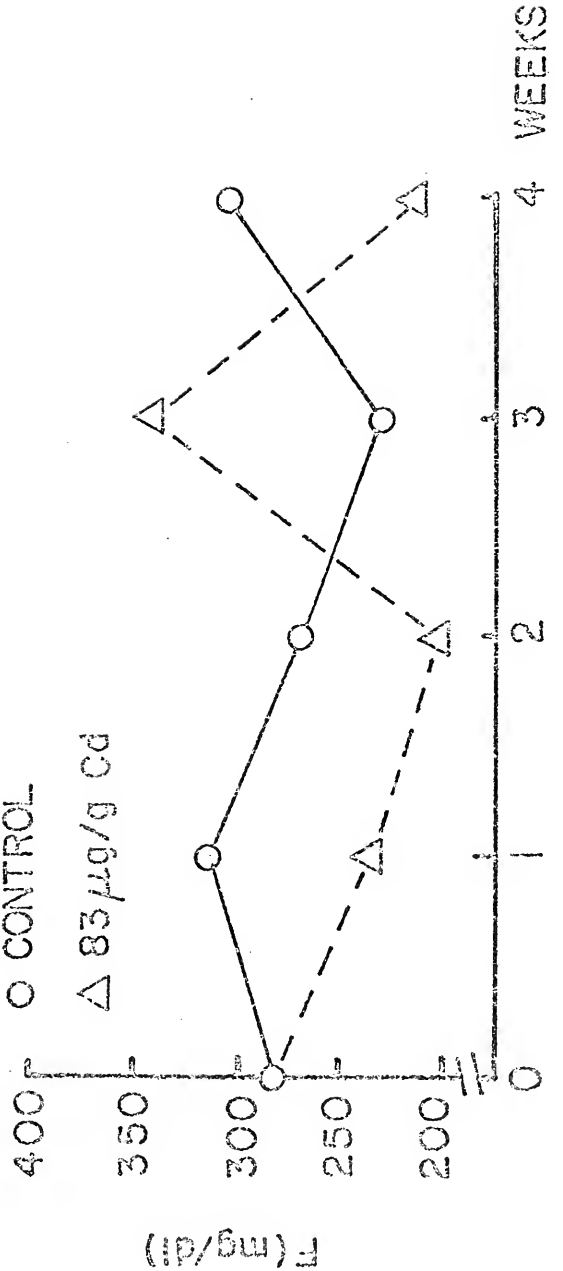

Figure 27. Mean weekly fibrinogen (F) concentrations in young pigs exposed to control or 83 µg/g Cd diets.

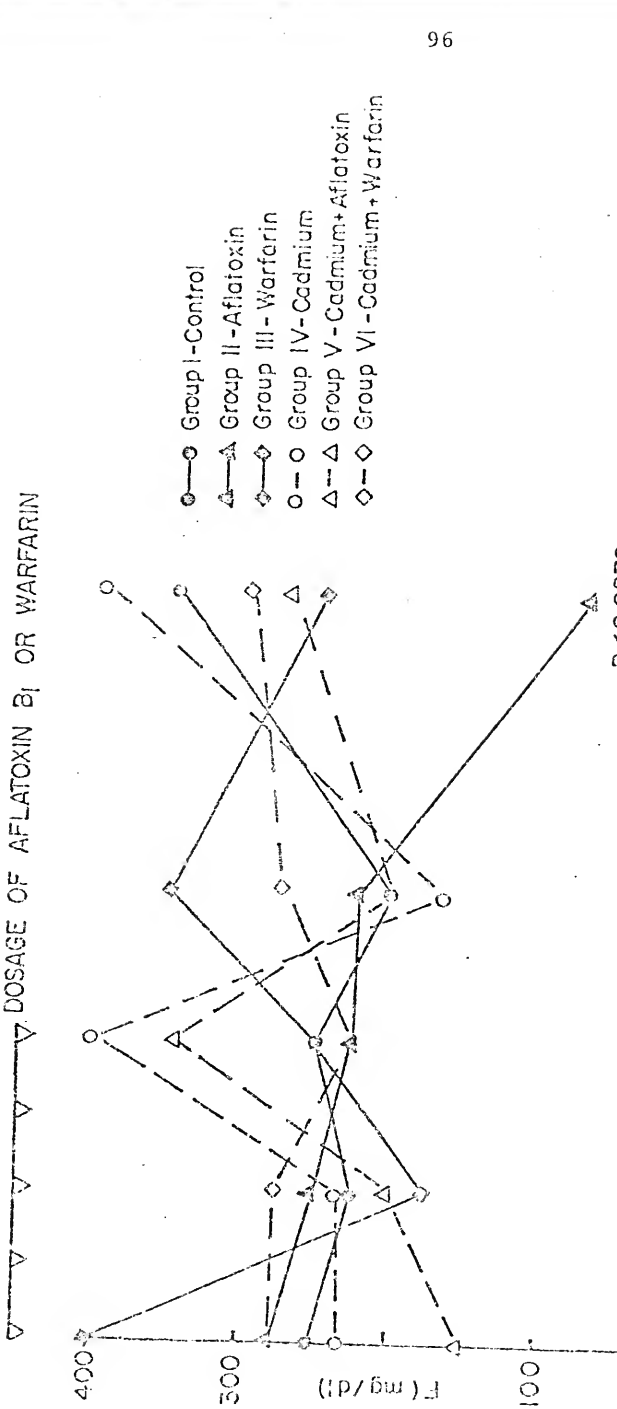

Figure 28. Mean fibrinogen (F) concentration in young pigs exposed to control or 83 µg/g Cd diets and treated with five daily oral doses of 0.2 mg/kg of aflatoxin B_1 or warfarin as indicated.

During the 10 days during and after the oral dosing with aflatoxin B_1, the pigs in group II (aflatoxin B_1 group) showed a significant decrease in F at the 10th day ($P < 0.0279$) of the treatment (Figure 28). On the other hand, no significant changes in F values were observed in group V (Cd + aflatoxin B_1 group).

During the 10 days after initiation of oral administration of warfarin, the F values were very variable in group III (warfarin group) but much less variable in group VI (Cd + warfarin group). No significant changes were observed in these two groups nor in the groups I (control group) or IV (Cd group).

When the blood coagulation factors determined in this experiment for the control groups (without Cd, aflatoxin B_1 or warfarin effect) are reviewed in the figures 23, 24, 25, 26, 27 and 28, the following normal ranges can be reported for young swine:

1. PT 12.65 ± 0.82 seconds
2. APTT 30.14 ± 3.40 seconds
3. F 268.8 ± 105.46 mg/100 ml

Serum Proteins

Total protein (TP): There were no significant changes in TP between the control and the 83 ug/g Cd diet groups (Figure 29) during the first 4 weeks of the experiment.

During and after initiation of the oral dosing of aflatoxin B_1 or warfarin, the only significant changes observed were in group II (aflatoxin B_1 group) where the values

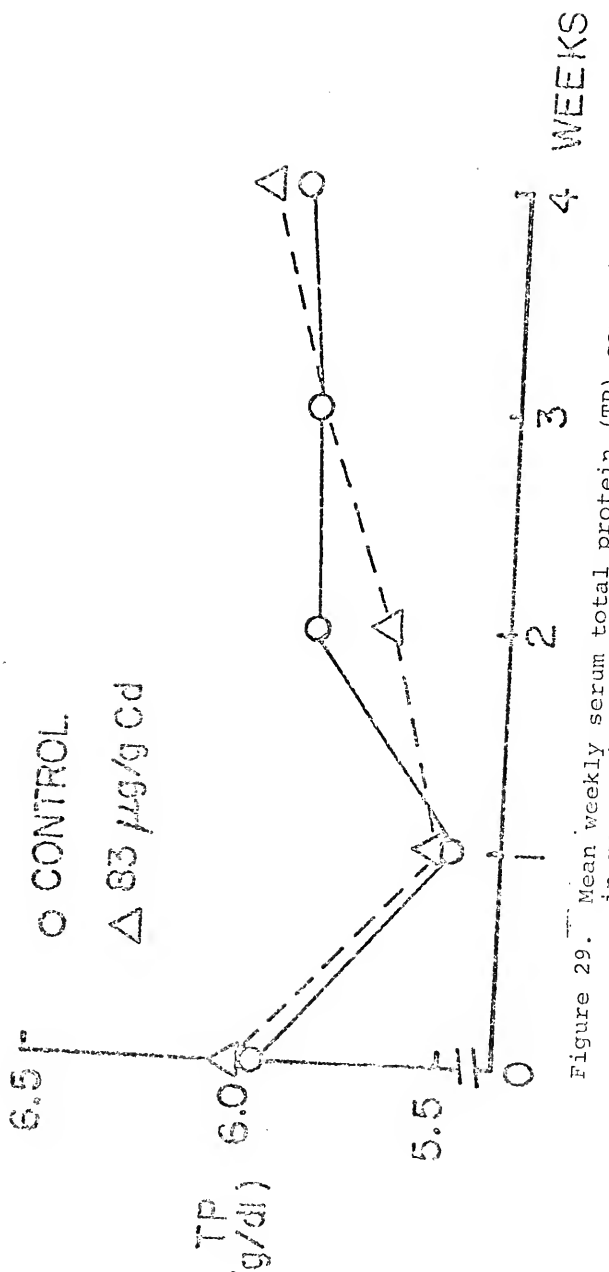

Figure 29. Mean weekly serum total protein (TP) concentrations in young pigs exposed to control or 83 µg/g Cd diets.

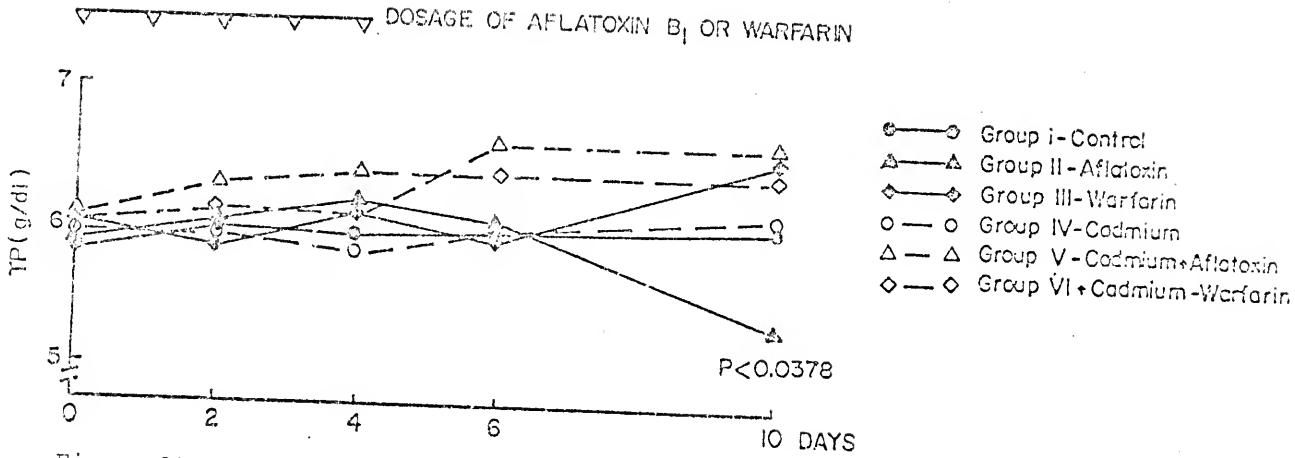

Figure 30. Mean serum total protein (TP) concentrations in young pigs exposed to control or 83 µg/g Cd diets and treated with five daily oral doses of 0.2 mg/kg of aflatoxin B_1 or warfarin as indicated.

decreased ($P<0.0378$) by the 10th day of the experiment (Figure 30). No significant changes are reported for groups I (control group), III (warfarin group), IV (Cd group), V (Cd + aflatoxin B_1 group) and VI (Cd + warfarin group).

Albumin (A): When all six treatment groups were evaluated, group II (aflatoxin B_1 group) showed the highest A levels and group IV the lowest levels at the 10th day of the critical phase of the experiment (Figure 31).

Alpha globulin (αG): When all of the six treatment groups are considered (Figure 32), group III was significantly lower at the 2nd day ($P < 0.0281$) and αG values for group II decreased steadily from day 4 to 10 of the critical phase of the experiment.

Beta globulin (βG): When data on all six treatment groups were reviewed (Figure 33), group II (aflatoxin B_1 group) levels decreased steadily and significantly from day 0 to 10. Significant differences were detected at the 4th ($P < 0.0478$), 6th ($P < 0.0063$) and 10th day ($P < 0.0019$) of the critical phase of the experiment.

Gamma globulin (γG): When data on all six treatment groups were evaluated (Figure 34), the γG levels were significantly lower in group IV (Cd group) at the 6th day ($P < 0.0198$) of the experiment; the group II γG levels (aflatoxin B_1 group) were significantly lower at the 10th day ($P < 0.05$).

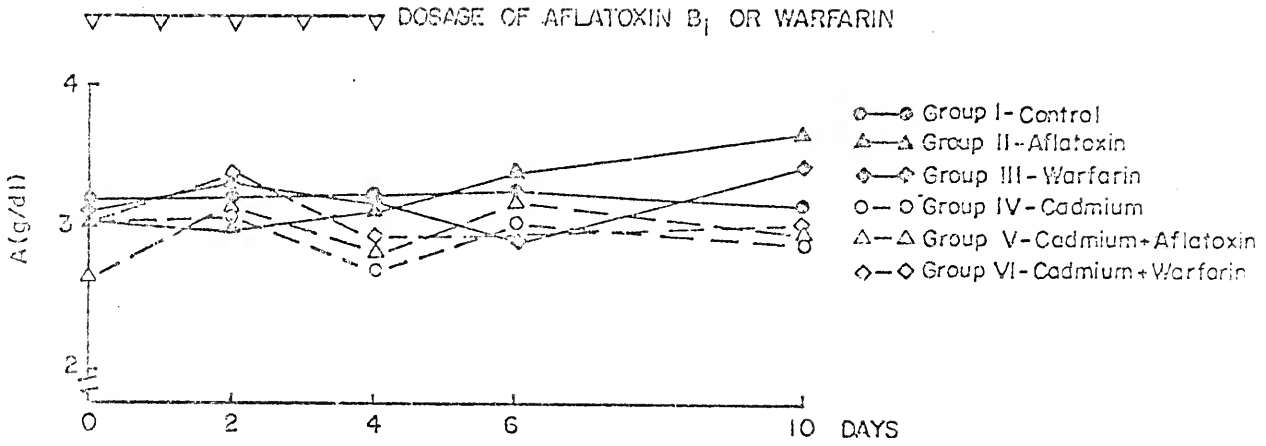

Figure 31. Mean serum albumin (A) concentrations in young pigs exposed to control or 83 µg/g Cd diets and treated with 5 daily doses of 0.2 mg/kg of aflatoxin B_1 or warfarin as indicated.

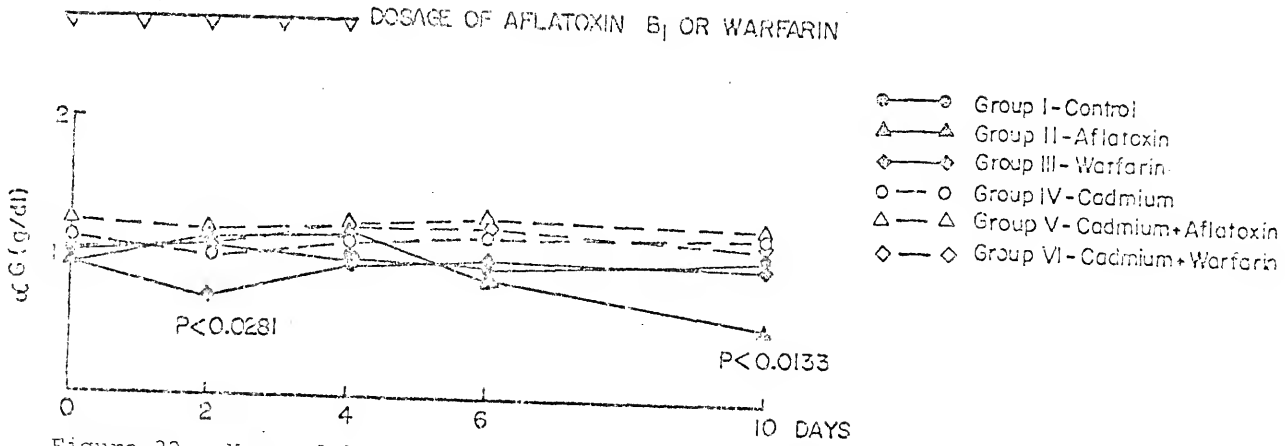

Figure 33. Mean alpha globulin (αG) concentrations in young pigs exposed to control or 83 μg/g Cd diets and treated with five daily doses of 0.2 mg/kg of aflatoxin B_1 or warfarin as indicated.

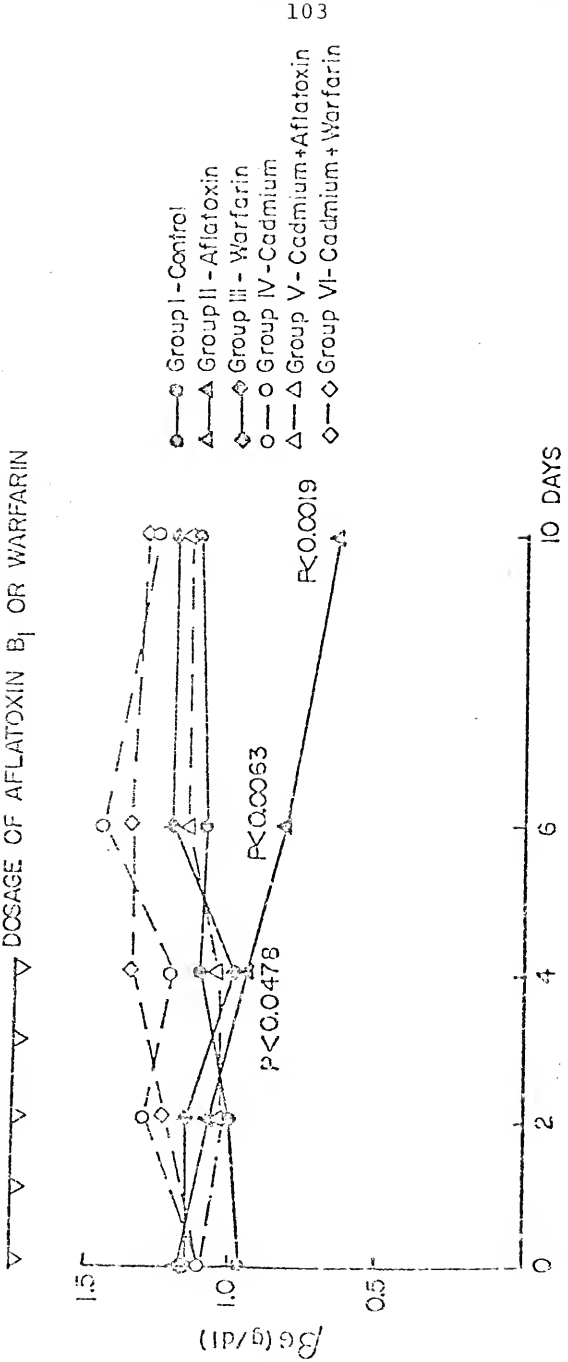

Figure 33. Mean beta globulin (βG) concentrations in young pigs exposed to control or 83 µg/g Cd diets and treated with five daily oral doses of 0.2 mg/kg of aflatoxin B_1 or warfarin as indicated.

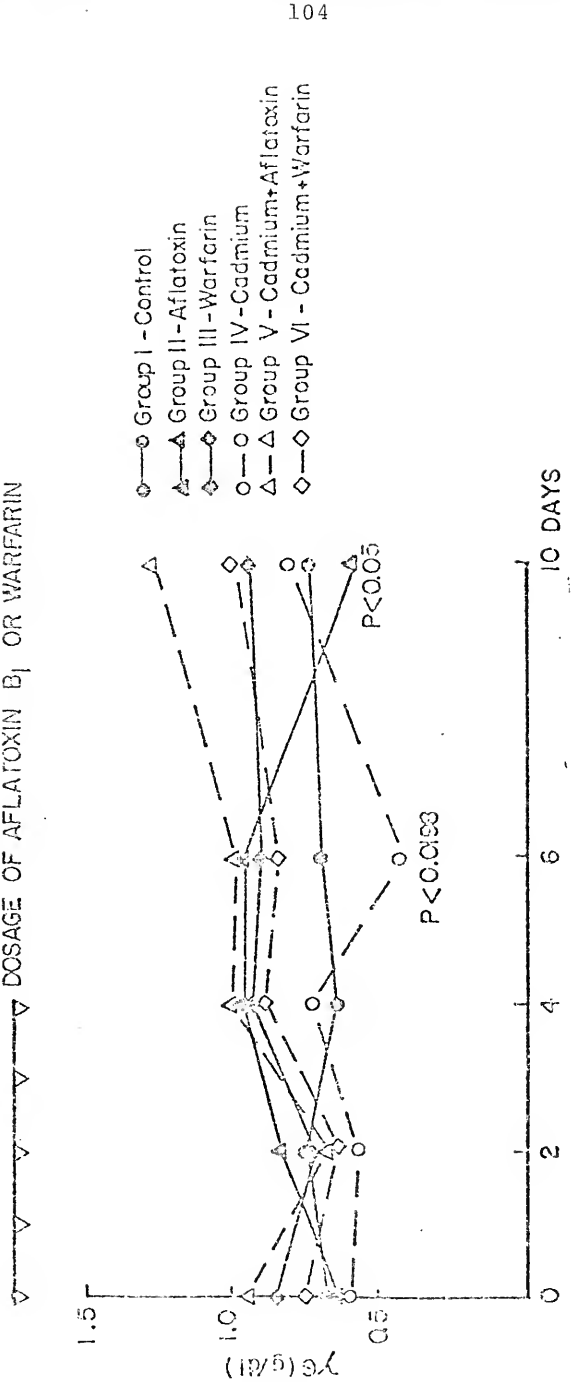

Figure 34. Mean gamma globulin (γG) concentrations in young pigs exposed to control or 83 µg/g Cd diets and treated with five daily oral doses of 0.2 mg/kg of aflatoxin or warfarin as indicated.

Metal Residues

At the time of the first slaughter after 4 weeks, Cd was at a higher concentration, both in the kidney ($P < 0.0054$) and liver ($P < 0.0003$) tissues, in the pigs consuming the 83 µg/g Cd diets as compared to those of the controls. No Cd was detected in the urine and very little (0.22 µg/g ± 0.01) in the muscle (Figures 35 and 37). Fe concentration was decreased in the kidney, liver and urine ($P < 0.0240$) in the pigs consuming the Cd diet (Figure 38). No significant differences between the concentration of iron were observed in the muscle tissue from either group (Figure 40). The Zn concentrations were significantly higher in the kidney tissues ($P < 0.0099$) and lower in the liver and muscle (Figures 41 and 43) in the pigs consuming the 83 µg/g Cd diet. The Cu concentrations were higher in the kidney of the Cd treated pigs (Figures 44 and 46). There were no significant differences in the concentration of Ca in the kidney, liver or muscle within the treatment group (Figures 47, 49).

At the second slaughter at the end of the experiment, Cd was accumulative and at a greater concentration in the kidney ($P < 0.0001$) and liver ($P < 0.0001$) in the pigs consuming the 83 µg/g Cd diet. No Cd was detected in the urine, when compared to levels present at the time of the first slaughter, or muscle of any group (Figures 36 and 37). Fe levels were significantly decreased in the liver ($P < 0.0073$) of the Cd treated pigs at the second slaughter. Fe levels were also lower in the kidney and urine ($P < 0.0131$) in the

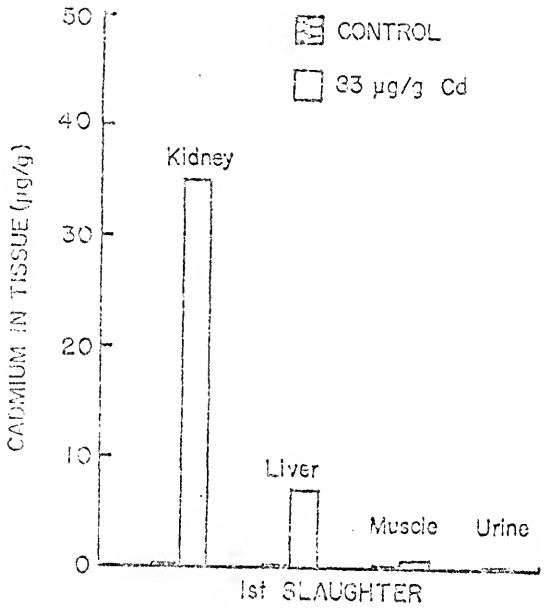

Figure 35. Average Cd concentration in the kidneys ($P < 0.0054$), liver ($P < 0.0003$), muscle ($P < 0.0099$) and urine at the first slaughter of control and 83 µg/g Cd diet treated weanling pigs.

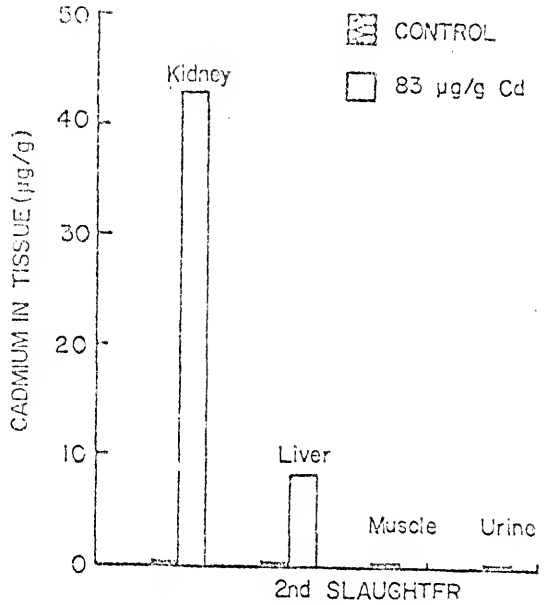

Figure 36. Average Cd concentration in the kidneys ($P < 0.0001$), liver ($P < 0.0001$), muscle ($P < 0.0027$) and urine at the second slaughter of control and 83 µg/g Cd diet treated weanling pigs.

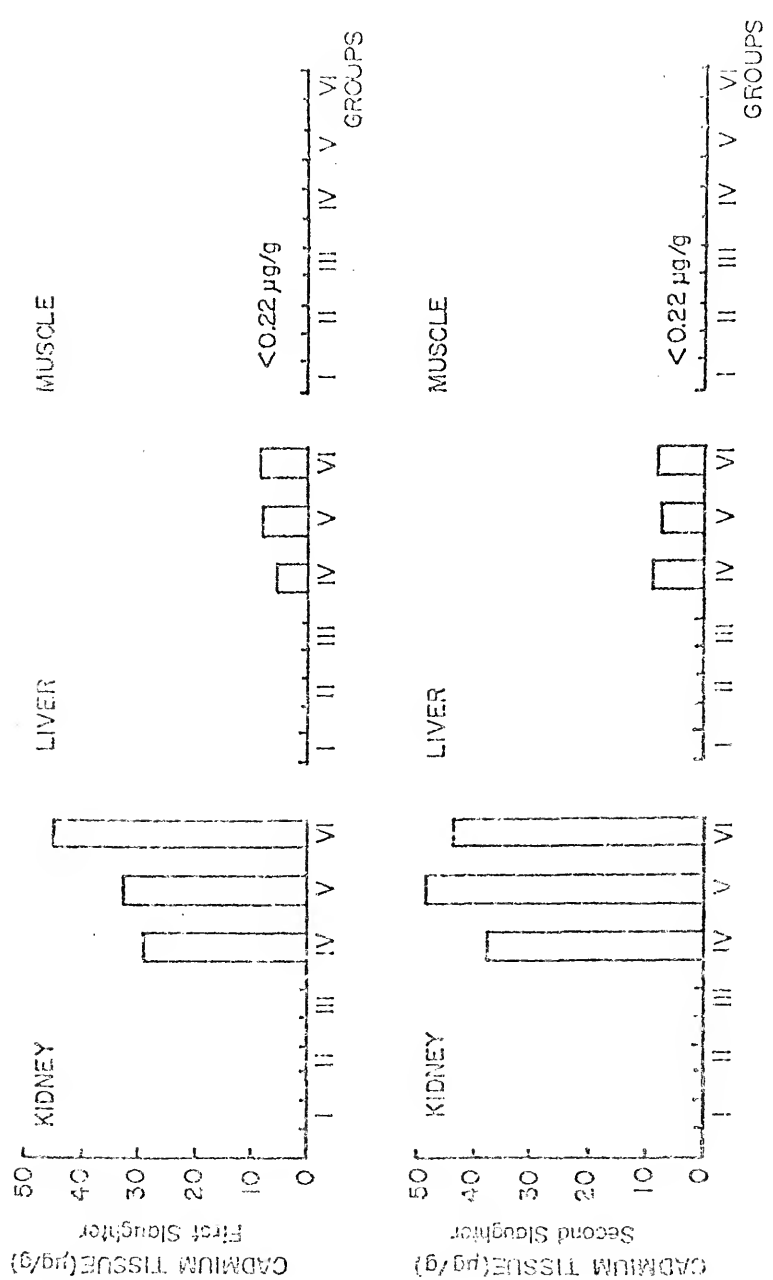

Figure 37. Group average values of Cd in tissues at the first and second slaughter.

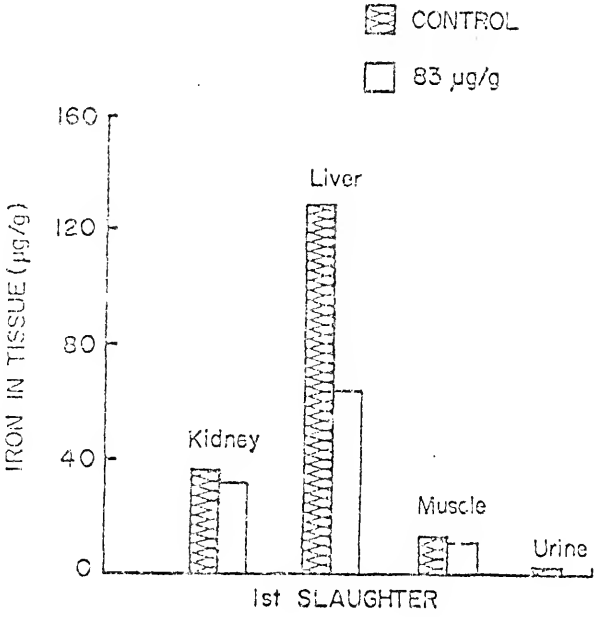

Figure 38. Average Fe concentration in the kidneys, liver, muscle and urine ($P < 0.0240$) at the first slaughter of control and 83 µg/g Cd diet treated weanling pigs.

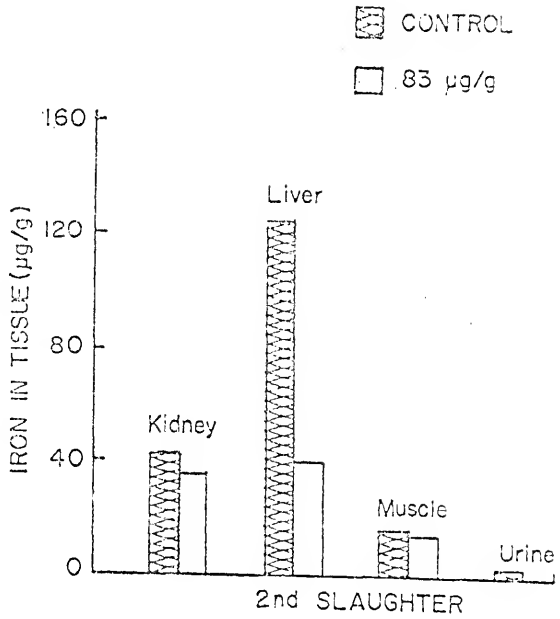

Figure 39. Average Fe concentration in kidneys, liver ($P < 0.0073$), muscle and urine ($P < 0.0131$) at the second slaughter of control and 83 µg/g Cd diet treated weanling pigs.

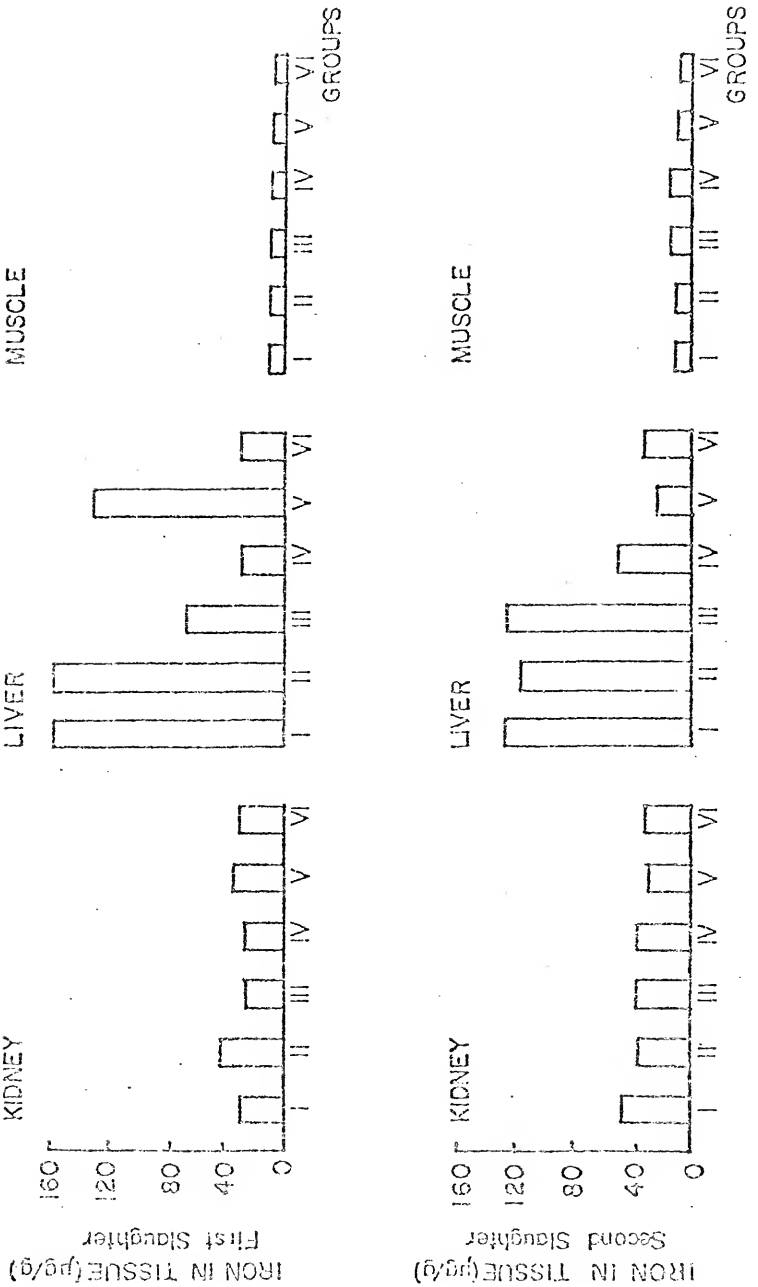

Figure 40. Group average value of Fe in tissues at the first and second slaughter.

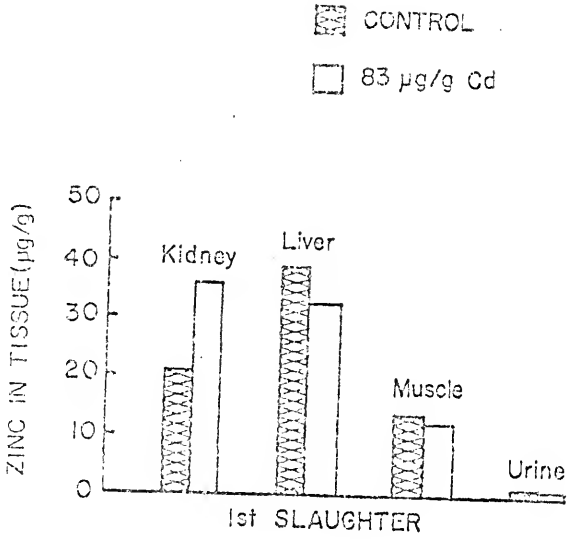

Figure 41. Average Zn concentration in the kidneys ($P < 0.0099$), liver, muscle and urine at the first slaughter of control and 83 µg/g Cd diet treated weanling pigs.

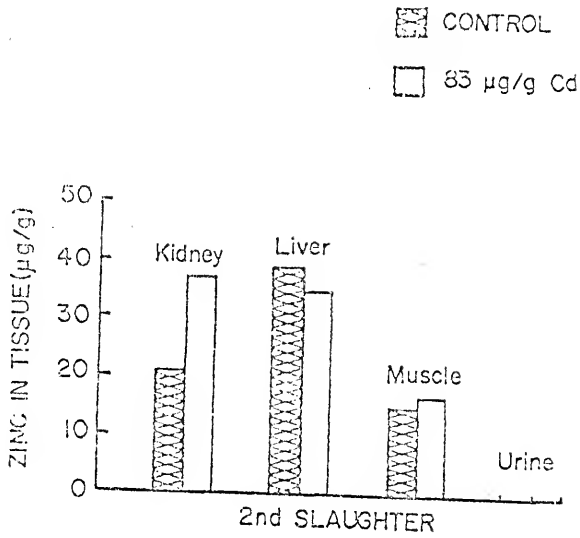

Figure 42. Average Zn concentration in the kidneys ($P < 0.0001$), liver ($P < 0.0025$) muscle and urine at the first slaughter of control and 83 µg/g Cd diet treated weanling pigs.

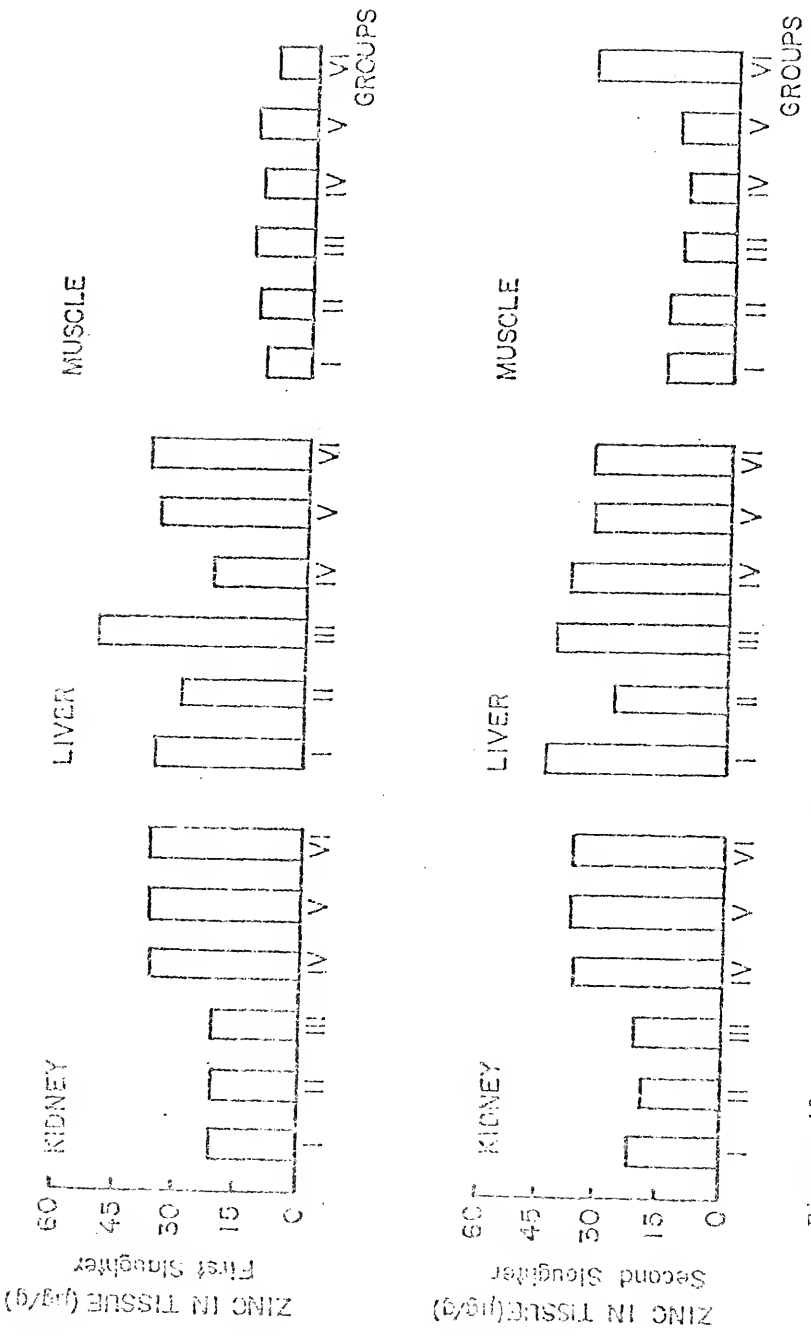

Figure 43. Group average value of Zn in tissues at the first and second slaughter.

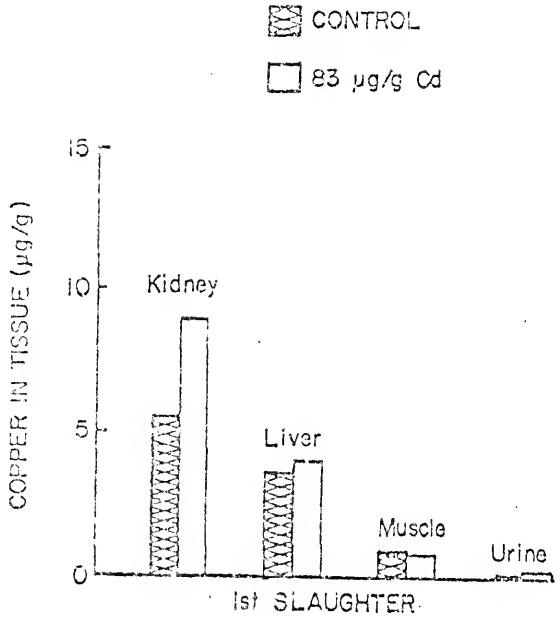

Figure 44. Average Cu concentration in the kidneys, liver, muscle and urine at the first slaughter of control and 83 µg/g Cd diet treated weanling pigs.

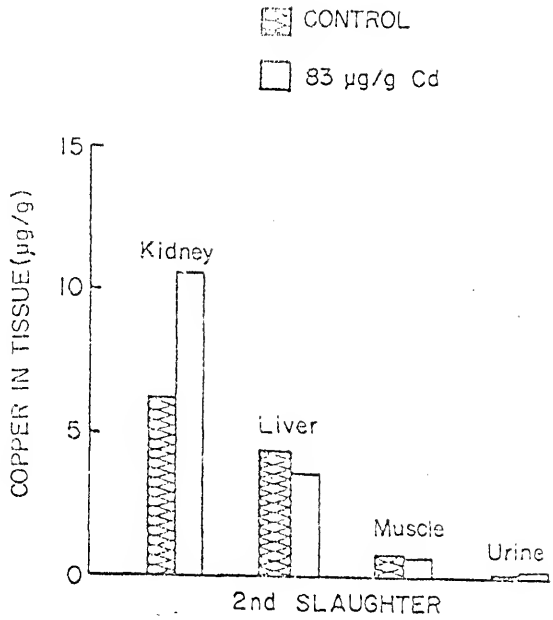

Figure 45. Average Cu concentration in the kidneys ($P < 0.0026$), liver ($P < 0.0025$) muscle and urine ($P < 0.0079$) at the second slaughter of control and 83 µg/g Cd diet treated weanling pigs.

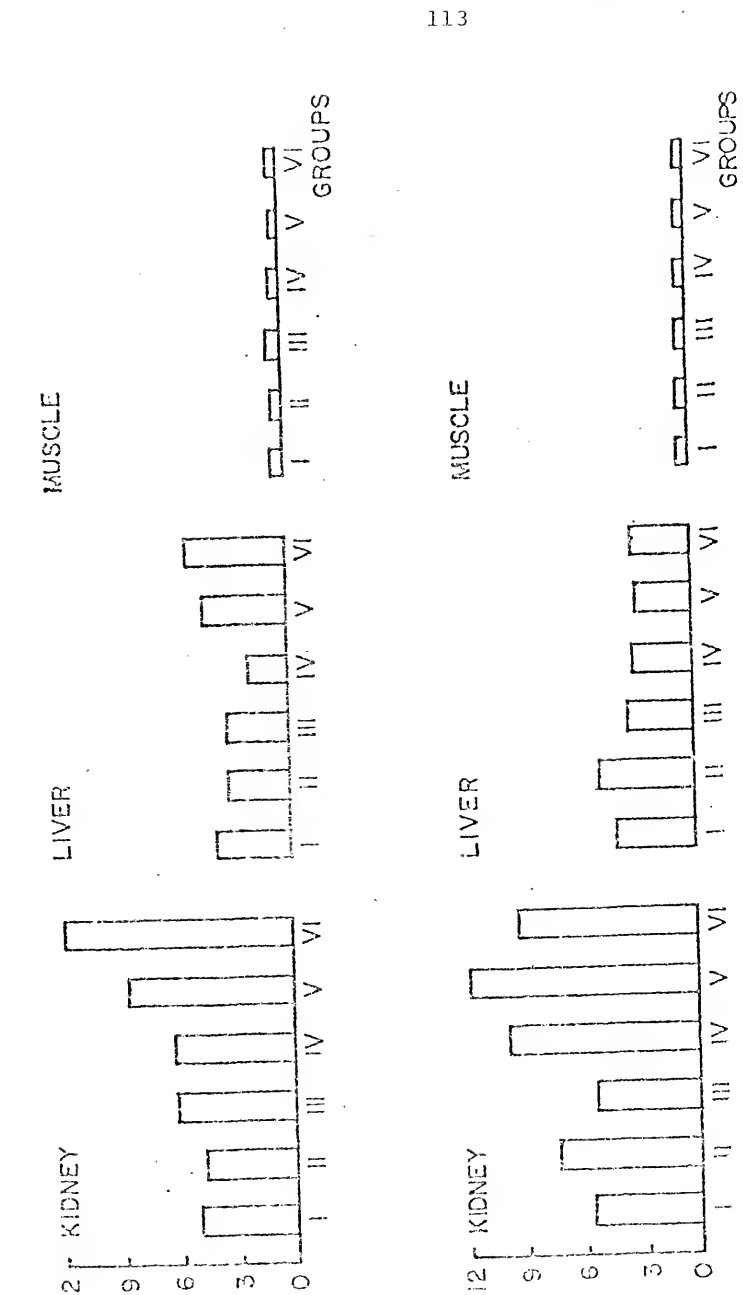

Figure 46. Group average value of Cu in tissues at the first and second slaughter.

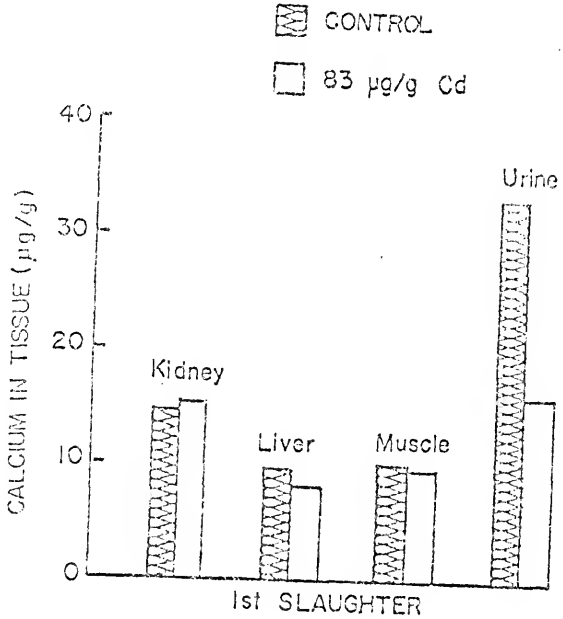

Figure 47. Average Ca concentration in the kidneys, liver, muscle and urine at the first slaughter of control and 83 µg/g Cd diet treated weanling pigs.

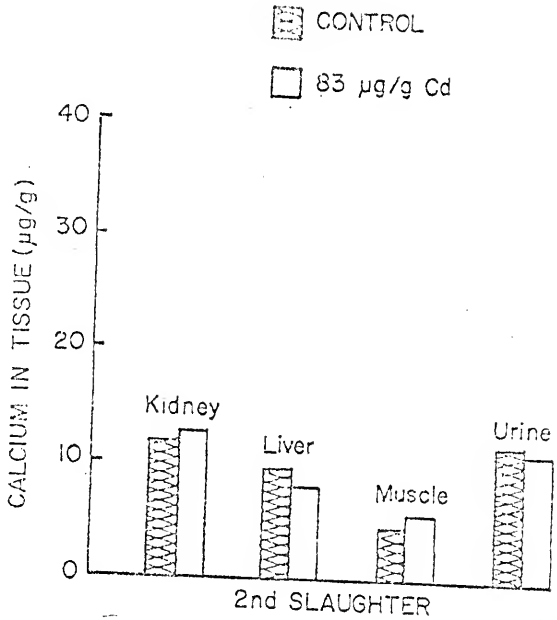

Figure 48. Average Ca concentration in the kidneys, liver, muscle and urine at the second slaughter of control and 83 µg/g Cd diet treated weanling pigs.

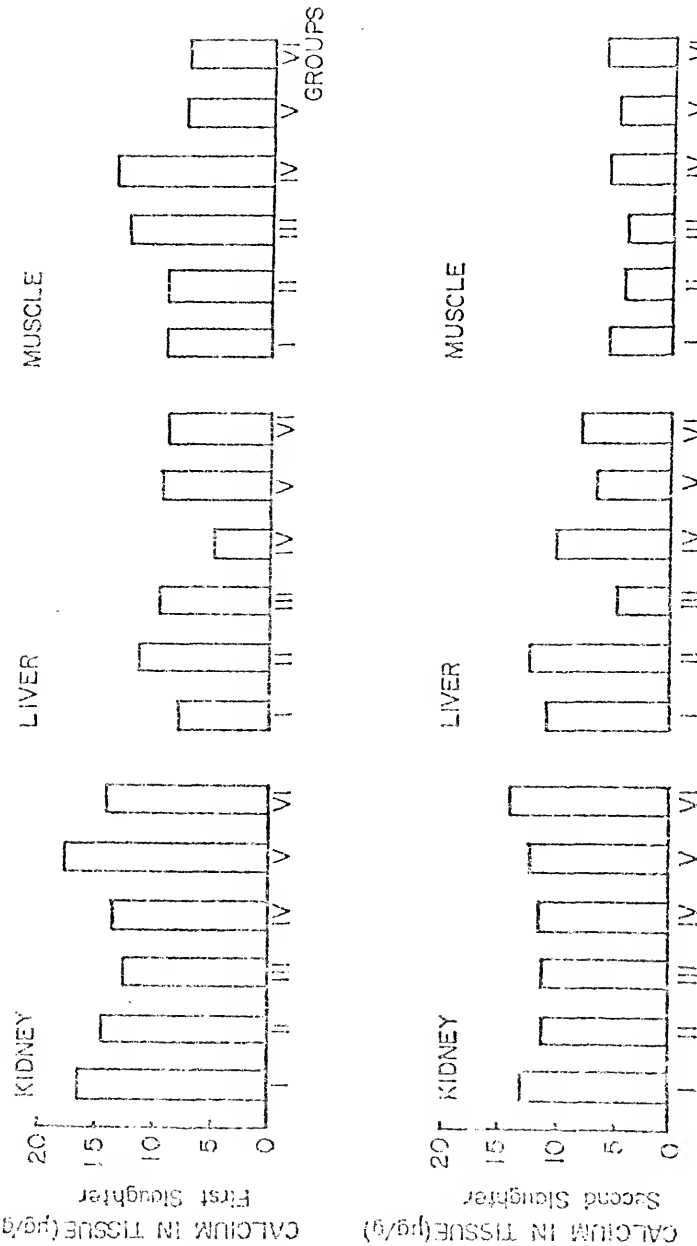

Figure 49. Group average value of Ca in tissues at the first and second slaughter.

pigs consuming the 83 µg/g Cd diet. No significant differences between the concentration of Fe in the muscles of either groups were observed (Figures 39 and 40). Zn levels were significantly higher in the kidney ($P < 0.0001$) but lower in the liver tissues ($P < 0.0025$) of the Cd treated group. Zn was also lower in the muscle tissues of the Cd treated pigs, except those from group VI (Figures 42 and 43). Cu levels were significantly higher in the kidney ($P < 0.0026$) but lower in the liver tissues ($P < 0.0025$) in pigs consuming the 83 µg/g Cd diet (Figures 45 and 46). No significant differences in the concentration of Ca in liver, kidney, muscle tissues or urine were observed between groups (Figures 48 and 49).

No significant changes in the concentration of Ca, Cd, Cu, Fe or Zn in the kidney, liver or muscle tissues were detected associated with treatment of pigs with aflatoxin B_1 or warfarin.

When the concentration of metal residues are considered in the control groups (Figures 35 to 49), the following ranges can be reported in tissues and urine for young swine in µg/g (wet basis):

Metal	Kidney	Liver	Muscle	Urine
Cd	0.00	0.00	0.00	0.00
Fe	30-48	70-160	10-14	0.10
Zn	20-23	30-50	12-16	0.3-1.3
Cu	5.0-6.5	3-5	0.75-0.86	0.05-0.10
Ca	12-16	9-12	5-10	12-33

Pathology

During the critical phase of treatment with aflatoxin B_1 or warfarin, different signs were observed among the groups. Pigs in group II (aflatoxin B_1 group) were depressed, with anorexia and no interest in feed or water. Animals presented a rough hair coat, as well as icteric mucous membranes. One animal presented rectal bleeding and swelling of the joints. The serum and urine of these animals were very icteric. The abdominal areas were very painful to external pressures. Three out of 4 animals were very sick at the end of the critical phase of the experiment.

Pigs in group III (warfarin group) remained alert and continued eating and drinking as usual during the treatment period. One animal was severely affected in the right hind leg due to possible intramuscular bleeding. However, all 4 animals were apparently normal at the end of the critical phase of the experiment.

Pigs in group IV (Cd exposed group), receiving the cadmium supplemented diet had blanched, pale mucous membranes. They were more sensitive to cold and huddled together over spilled feed to decrease body heat loss.

Pigs in group V (Cd + aflatoxin B_1 group) were not as severely affected as those in group II. The hair coat appeared normal. The animals were not depressed although three out of 4 presented yellow, icteric serum at necropsy. At the end of the critical phase of the experiment, they appeared normal.

Pigs in group VI were more adversely affected by warfarin (Cd + warfarin group) than group III. Blood continued dripping for more than 1 minute after withdrawing the needle. Extensive subcutaneous hemorrhages in the neck, as well as in the ham areas, were observed in one case. Two out of 4 animals died by the 10th day of the treatment.

At necropsy, the most outstanding gross lesions were presented by pigs in groups II and VI. Both kidneys and livers, as well as the perineal and intestinal fat, were very icteric in 3 of 4 pigsin group II. The thoracic cavity of one animal that died suddenly was blood-filled in group VI.

Animals treated with Cd presented pale livers, kidneys and muscles.

Pathologic examination of kidneys, livers and muscles collected at the first slaughter and prior to dosing with aflatoxin B_1 or warfarin, revealed only minor lesions. The tissues from the control pigs (including the pigs from pen I, II and III as one control group) were normal, except for occasional areas of interstitial lymphocytic nephritis presented by 3 of 6 pigs. There was granular appearance, particularly in the periphery of the hepatic lobules, due to glycogen deposition in 2 out of the 6 animals. The tissues from the 83 µg/g Cd diet treated group (including the pigs from pen IV, V and VI as one Cd group) were normal, except for occasional areas of interstitial lymphocytic nephritis presented by 4 of 6 pigs. There were granular hepatic changes in the periphery of the hepatic lobules due to glycogen

deposition in 2 out of the 6 pigs. One may conclude that the condition of the pigs, based on histopathologic evaluation, were very similar at this stage of the experiment.

Pathologic examination of kidneys, livers and muscles from the 6 groups of pigs at the second slaughter after treatment with cadmium, aflatoxin B_1 and/or warfrain revealed the following lesions:

1. Group I (control group): The tissues were normal. In one liver, the sinusoids in the area of the central vein were slightly dilated and in another, an occasional area adjacent to the portal triads contained small accumulations of lymphocytes. A kidney from one animal in this group presented an interstitial lymphocytic nephritis in both the cortex and medulla. The muscles were normal.
2. Group II (aflatoxin B_1 group): Three out of 4 livers were severely affected by aflatoxin B_1. There was general vacuolization of hepatocytes and bile duct proliferation, principally in the periportal area, but also generally throughout the lobule. Severe, fatty degenerative changes occurred in every lobule. There was also some dilation of the central veins and hemorrhage, along with a few lymphocytes. In one animal's kidney, there were necrotic changes of the epithelium of the proximal convoluted tubules, along with hemorrhage. In another kidney, there was interstitial nephritis in the medulla.

3. Group III (warfarin group): Kidneys and muscles of this group were normal. In 3 of the 4 livers, there was some dilatation of the sinusoids in the central vein areas.

4. Group IV (Cd group): The tissues in this group revealed minor lesions. All kidney and muscle sections were normal. In 2 of the 4 livers, the cells nearest the portal triad were richer in glycogen. One liver presented some mild dilatation of the sinusoids in the central vein area. One pig died in this group at the beginning of the 4th week of the experiment. Cardiac, splenic and pancreatic tissues were normal. There was necrosis of the tips of the villi of the small intestine. The alveoli of the lung were partially collapsed because of capillary congestion, along with interlobular edema. There was also some fluid in alveoli, but this was rare. The alveolar septal walls were thickened, apparently due to the infiltration by lymphocytes.

5. Group V (Cd + aflatoxin B_1 group): Kidney and muscle sections were normal. Three of the 4 livers had undergone hydropic degeneration of the lobular peripheral cells. The sections were lightly stained and were apparently rich in glycogen.

6. Group VI (Cd + warfarin group): In 3 of the 4 pigs, there was marked variation in size and shape of bundles of muscle fibers sectioned, and in some cases,

the fibers were widely separated. In fact, this was quite an unexpected observation. There were no degenerative changes, however. In one of the kidneys, some cirrhosis in the cortex, along with fatty degeneration of the epithelium of the collecting tubules, was observed (or perhaps the distal convoluted tubules had undergone fatty changes). In 2 of 4 livers, there were cells rich in glycogen and the sections were lightly stained. In one liver, there were mild fatty changes in the hepatocytes.

The urine samples, on assay, were negative for proteins, glucose, bilirubin and ketones. Most of the specific gravities of the different urine samples, as well as those of the control group, were from 1.020 - 1.035.

One animal treated with aflatoxin B_1 alone presented a low urine specific gravity of 1.008 with a pH of 5. The histopathologic kidney studies of this animal revealed necrotic changes in the epithelium of the proximal convoluted tubules, along with hemorrhage. The pH of the different urine samples ranged from 5 - 7.

Mycotoxins

All rations were tested weekly for aflatoxin B_1 and contained less that 20 ng/g. A tolerance or guideline level of 20 ng/g has been established for feeds and foods intended for interstate shipment by the FDA (Edds, 1979).

Microbiology

Feeds were examined for animal pathogens on the first and last day of the experiment, but were negative in all cases.

Leptospiral titers were negative in all 36 pigs prior to dosing.

Parasitology

The fecal samples were free of parasitic ova other than coccidia, which were only found during the pretrial week in all groups.

Pesticides

Feeds were submitted for pesticide analyses on the first and last day of the experiment and found negative to organochloride, organophosphate and carbamate insecticides.

CHAPTER V

DISCUSSION

The purpose of this experiment was to compare the toxic effects of aflatoxin B_1, a dihydrofuranocoumarin, and warfarin, a 3-(α -acetonyl-benzyl)-4 hydroxycoumarin; and also to determine whether an additive effect from either aflatoxin B_1 or warfarin occurs when Cd is present daily in the diets of young pigs.

This is an unique experiment in which three factors that frequently accumulate or alter liver function, were analyzed for possible additive effects.

The toxic properties of aflatoxin B_1 differ, depending on the dose and the duration of exposure (Newberne et al., 1969). Susceptibility to aflatoxins also appears to vary with species, breed, sex, age, nutrition, health status, liver diseases and parasitic diseases (Osuna et al., 1977; Osuna and Edds, 1976). These same factors seem to affect the toxic properties of warfarin (Deckert, 1974). Indeed, the anticoagulant rodenticides are more toxic when ingested daily over a period of 5 - 7 days. Multiple small doses are 5 - 100 times as toxic as the same single dose, depending on the species (Hatch, 1977). Clinical complications have also arisen as a consequence of warfarin interactions with other medications (Fasco et al., 1978).

Therefore, five daily oral doses of aflatoxin B_1 or warfarin were given to young susceptible pigs (Edds, 1979; Osweiler, 1978) in order to compare the toxic properties of these two coumarin related chemicals at low multiple dosages with or without the presence of added cadmium in the diets.

It was demonstrated that daily ingestion of 0.2 mg/kg of warfarin for 7 days could be lethal in young pigs (Osweiler, 1978). No information was available at the time of design of this experiment about the toxic properties of repeated doses of aflatoxin B_1 at 0.2 mg/kg in young pigs. However, it has been reported that the acute, single dose LD_{50} of aflatoxin B_1 in young pigs was 0.52 mg/kg of body weight (Hatch, 1977) and 1.0 - 2.0 mg/kg of body weight (Edds, 1979; Cysewski et al., 1968). So, it was thought that a daily dose of 0.2 mg of aflatoxin B_1/kg of body weight for 5 days could also be highly toxic, based on the earlier reports.

Intestinal absorption of warfarin is known to be complete (Clatanoff et al., 1954; Shapiro and Ciferri, 1957). Plasma levels of warfarin were found to be identical following oral or intravenous administration (O'Reilly et al., 1963). No warfarin was found in the stool after large oral doses (400 mg). The degree of absorption of oral aflatoxin B_1 in diets is not known. However, the data in young pigs indicated that it is absorbed quite readily with toxic levels producing enzymatic liver changes 3 hours after ingestion and being marked at 6 hours (Cysewski et al., 1968).

Generally, Cd concentrations are highest in the kidney, followed by the liver (Friberg et al., 1971). Liver and renal function influence both the intensity and the duration of response of oral anticoagulants (Deckert, 1974). Cadmium has been found in lung and other tissues in cigarette smokers (Menden et al., 1972) and significant increases of Cd were present in human kidney and liver tissues that had emphysema and carcinoma of the lungs (Flick et al., 1971). One may conclude that research describing the interactions of Cd with anticoagulants could be of importance, since clinical complications of patients treated with coumadin derivatives have arisen with administration of other medications (Fasco et al., 1978).

Recently, studies on the use of animal waste and sewage sludge as an ingredient in animal feeds have been of interest. One lot of sewage sludge from Chicago was secured for soil treatment trials, grain production and animal health effects studies which was found to contain high levels of Cd (165 µg/g). An earlier trial compared the effects of feeding weanling pigs a standard swine starter ration, and one containing 50% dried, activated, Chicago sewage sludge (containing 165 µg/g Cd) vs. an 18 percent protein starter ration containing 83 µg/g Cd for 9 weeks (Osuna et al., 1979). Cd concentrations in the liver of pigs at slaughter of the Cd supplemented diet group (12.98 µg/g) were 4 fold the amount found in the Chicago sludge diet group, but no pathologic lesions were observed in the controls or either of the cadmium supplemented diets.

The purpose of again using the 83 µg/g Cd diet in pigs was to determine whether animals may be predisposed to higher risk when exposed to moldy feed containing aflatoxin B_1 or other poisons such as rodenticides, even if no histopathologic liver lesions were induced in pigs exposed to Cd diets. This research was designed to expose groups of pigs to Cd diets for 4 weeks before they were treated with aflatoxin B_1 or warfarin, since it had been demonstrated that Cd would accumulate in highly significant concentrations (12 µg/g) by the 4th week, concurrent with an extreme microcytic hypochromic anemia (Osuna et al., 1979).

Finally, this critical phase of the experiment included slaughter of the pigs after the 10th day in order to collect tissues to assay for metal content and pathologic evaluations.

Performance

Depressed growth and feed consumption were evident in pigs consuming 83 µg/g Cd diets (Figure3 and Table 3). It is well known that growth depression and anemia are caused by Cd ingestion (Fox et al., 1971; Cousins et al., 1973; Sansi and Pond, 1974; Maji and Yoshida, 1974; Suzuki and Yoshida, 1978; Osuna et al., 1979). Suzuki and Yoshida (1978) suggested that the growth retardation induced by dietary 50 µg/g Cd is mainly due to the reduced feed consumption.

The non-metallothionein bound Cd competes and displaces essential cations from enzymes and nucleic acids, thus affecting their physiological function and causing toxicity (Vallee and

Ulmer, 1972; Shaikh and Smith, 1976) which affects animal performance. This is also associated with a decrease in feed efficiency in the Cd diet treated group (Table 3).

The decrease in rate of growth in groups II (aflatoxin B_1 group) and V (Cd + aflatoxin B_1 group) was associated with a decrease in feed consumption (Edds, 1979; Cysewski et al., 1978) and with decreased protein synthesis resulting from aflatoxicosis (Osuna et al., 1977). Other signs, in addition to the decrease in feed consumption in group II, were depression with loss of appetite and lameness.

Cadmium apparently provided some protection in pigs in group V, since there were only minor decreases in mean body weights. Pigs in group II were losing 0.34 kg of BW per pig per day, while those in group V were losing 0.05 kg per pig per day (Figure 4 and Table 4). The explanation for this Cd protection against aflatoxicosis seems to be related to inhibition of the activation of aflatoxin B_1 into a toxic metabolite by the hepatic microsomal enzyme system.

The decreased rate of growth and mean body weights in pigs in groups III (warfarin group) and VI (Cd + warfarin group) during the critical phase can be associated with both lameness and hemorrhagic enteritis (Osweiler, 1978). Animals in group VI were more severely affected than those in group III (Figure 4 and Table 4). Because of severe lameness and subcutaneous hematomas in the ventral surface of the neck and jaws in pigs of group VI, the animals ate less and gained at

a slower rate than those in group III. The additive toxic effect of Cd and warfarin increased the toxicity of warfarin in group VI.

Hematology

An extreme microcytic hypochromic anemia (Figures 5, 7 and 9) was observed again in young growing pigs when fed 83 µg/g Cd diet by the 4th week of the experiment (Osuna et al., 1979). Although inhibition by Cd of iron metabolism is probably due to direct competition between these cations for an intestinal mucosal binding site other than thionein (Hamilton and Valberg, 1974), an indirect response, i.e., secondary to the primary interactions between Cd and Cu or Zn, as suggested initially (Hill et al., 1963) also remains possible (Stonard and Webb, 1976). The anemia and the interactions of Cd with Fe, Cu or Zn are well supported by the tissue metal residues reported in this trial (Figures 37 - 46). A loss of iron from liver and kidneys also was observed following dietary Cd treatment as described by others (Bun and Matrone, 1966; Stonard and Webb, 1976; Osuna et al., 1979).

Lowering of WBC counts (Figure 13) in the Cd diet treated group, with decreased resistance to infection, may explain the death and pneumonia of one pig in group IV. It is also important to associate the extreme anemic condition of the animals and the leucopenia detected by the 4th week of the experiment in the Cd treated group. One may suggest that because of the lowering of resistance in the pigs on the

cadmium supplemented diet rendered them more susceptible to infectious and parasitic diseases.

Aflatoxin B_1 dosage induced high values of RBC, PCV and Hb in group II at the 6th day of the critical phase of the experiment (Figures 8, 10 and 12) which may be associated with pyrexia or lower water intake (Cysewski et al., 1968; Sisk et al., 1968). The abrupt drop in Hb, PCV and RBC values in group II by the 10th day of this phase of the experiment may have been associated with the high increase in PT and APTT (Figures 24 and 26) in the same pigs.

There was a protective effect of Cd against aflatoxicosis as demonstrated by animals in group V (Cd + aflatoxin B_1 group). The signs of hemoconcentration were not significant by the 6th day and no dramatic drops in the Hb, PCV and RBC values were observed by the 10th day of the critical phase of the experiment.

Serum Enzymes

Alkaline phosphatase (AP): The various alkaline phosphatase (AP) isoenzymes are ubiquitous throughout the body, although they are primarily present in high concentrations in liver, bone, intestine, kidney, placenta and white blood cells (Tietz, 1976). Rapid growth of pigs in the control group (Figure 3) may explain the increased values of AP from the beginning through the 4th week of the experiment (Figure 15) due to bone marrow proliferation. On the other hand, the pigs in the Cd diet-treated group had slower weight gains and lower WBC counts than the control group with decreasing AP values with time.

The aflatoxins, a group of closely related metabolites of Aspergillus flavus, are well known to be potent hepatotoxins (Wogan, 1968; Edds, 1973; Newberne, 1973; Hatch, 1977). The signs and lesions of aflatoxicosis in calves included stunting, icterus with hepatic necrosis, hemorrhage and bile duct hyperplasia, along with hydropic and fatty degeneration of hepatocytes (Osuna et al., 1977; Edds and Osuna, 1976).

Some AP is normally excreted in the bile and the serum activity of AP is increased with interference with bile flow. It has been suggested recently that the response of liver to any form of biliary obstruction is to synthesize more AP. Values indicating cholestasis are usually well above the levels asscciated with non-hepatic disorders. Intrahepatic obstruction of the bile flow also raises serum AP, but usually to a lesser extent (up to 2.5 times the upper normal limit) than the extrahepatic one (10 - 12 times the upper normal limit) (Tietz, 1976).

The significant increased values of AP in group II (aflatoxin B_1 group) can be related with the hepatotoxic effects of aflatoxin B_1 (Figure 16). The necropsy and histopathologic results support the hepatic origin of the AP levels in this group. That is, there were icteric conditions of the liver and mucous membranes, general vacuolization of hepatocytes, principally in the periportal area and severe fatty degeneration throughout every lobule. The rise in AP values can be associated with intrahepatic obstruction of the bile flow (Tietz, 1976) since they were about 3 times above the upper, normal or control values by the 4th day of the critical phase of the experiment.

The mean AP values in pigs in group V (Cd + aflatoxin B_1 group) increased moderately during and after dosing with aflatoxin B_1. The histopathologic lesions and the serum enzyme values found suggest that aflatoxin B_1 was absorbed from the intestinal wall and affected the livers of pigs in this group. However, aflatoxin B_1 did not produce as severe lesions or enzyme changes in group V as compared with those of group II. Apparently, the presence of Cd decreased the toxicity of aflatoxin B_1 to the hepatocytes.

Sorbitol dehydrogenase (SDH): The usefulness of the SDH test has been confirmed recently by many investigators as an assay for hepatocyte damage in most domestic animals, including pigs. One advantage of using SDH as a measure of the severity of necrosis is that only this one enzyme test would be needed for all species. A disadvantage of the SDH test is that its activity in serum declines appreciably after 24 hours storage, even under refrigeration, and serum samples must be analyzed within 12 hours. In acute hepatitis, the levels of SDH increase 10 - 30 times above the normal level. However, in chronic, stabilized hepatitis, as well as cirrhosis and obstructive jaundice, enzyme activity, after the initial rise, falls to normal or, at most, stays marginally elevated (Coles, 1967; Tietz, 1976).

One may conclude that aflatoxin B_1 produced direct necrosis of the hepatic cells (Figure 18). There were acute hepatotoxic effects with SDH values 10 - 30 times above the normal level by the 2nd day of the critical phase of the experiment. As

described by Tietz (1976), after the initial rise, SDH falls to normal or near normal, as in obstructive jaundice. The inflamed cells may have occluded some of the medium and small size bile ducts affecting the rate of bile flow (Figure 16).

The mean SDH values in pigs in group V (Cd + aflatoxin B_1 group) suggest that aflatoxin B_1 was absorbed from the gastrointestinal tract and produced less hepatic cell damage, but at the same time as occurred in those in group II (Figure 18).

The mean SDH values were higher in the pigs of the Cd diet treated group than those of the control pigs through the 4th week (Figure 17). These higher values could have resulted from minor liver damage induced by Cd. However, the greater concentrations obtained in the Cd diet group pigs during these first 4 weeks were not associated with any major clinical signs or histopathologic changes.

Aspartate Aminotransferase (SGOT). Elevations in the activity of SGOT are associated with cell necrosis of many tissues. Pathology involving the skeletal or cardiac muscle and/or the hepatic parenchyma allows for the leakage of large amounts of this enzyme into the blood (Cornelius et al., 1959 and 1963). SGOT levels, however, can be used prognostically to evaluate the degree of liver necrosis, once it has been established that other non-hepatic diseases are absent (Cornelius et al., 1959 and 1963).

Cysewski et al. (1968) produced acute aflatoxicosis in young pigs, 11.4 - 21.4 kg average body weight, by giving single doses of aflatoxin B_1 at 1.98 mg/kg. Altered liver

function was detected at 3 hours and was marked at 6 hours. SGOT and ornithine carbamyl transferase activities were elevated markedly after 6 - 9 hours.

The SGOT levels observed in cirrhosis vary with the status of the cirrhotic process, ranging from upper normal to some 4 - 5 times normal. Slight or intermediate elevations are also seen in obstructive jaundice (Tietz, 1976).

Elevations of mean SGOT levels in groups II (aflatoxin B_1 group) and V (Cd + aflatoxin B_1) can be associated with alterations in the hepatic parenchyma as induced by aflatoxin B_1 (Figure 20). Altered liver function, liver necrosis, hepatic vacuolation, icterus and bile duct proliferation have been described in pigs with aflatoxicosis (Edds, 1979; Hayes et al., 1978; Cysewski et al., 1968; Sisk et al., 1968; Annau et al., 1964; Harding et al., 1963; Gumbmann and Williams, 1969).

Changes in serum enzyme levels (AP, SDH, SGOT) and liver function tests (TP, PT, APTT, F) as described before are indicative of marked hepatic damage involving destruction of the normal hepatic architecture as described by others (Annau et al., 1964; Edds, 1973; Osuna et al., 1977). The histopathologic lesions found in groups II and V are correlated with the enzymatic changes associated with liver damage. The SGOT values observed in group II were about 5 times ($P < 0.05$) those in the normal control (group I). Pigs in group II were more severely affected than those of group V as indicated by the higher SGOT values (Figure 20).

Elevation in the activity of SGOT in the warfarin group (group III) by the 2nd day of the critical phase was also observed ($P < 0.05$) and can probably be associated with pathology involving the skeletal muscle due to intramuscular bleeding and lameness.

Blood Urea Nitrogen (BUN): The determination of serum urea nitrogen is presently the most widely used screening test for evaluation of kidney function (Tietz, 1976).

Proteinuria is considered to be the first sign of renal tubular dysfunction and is said to occur when renal cortical levels of Cd reach about 200 µg/g (wet weight) compared to normal levels of about 50 µg/g in adults (Friberg et al., 1971). Stop-flow analysis, performed at the end of successive Cd administrations, revealed proximal dysfunction, as well as glomerular and distal tubular dysfunction (Nomiyama et al., 1973).

No significant differences between the BUN values of the control and the 83 µg/g Cd diet treated groups during the first 4 weeks were observed (Figure 21). The urine samples of the pigs from the Cd diet treated group were negative for protein, glucose, bilirubin and ketones. The specific gravities of the urine samples of the Cd diet treated pigs were in the same normal range as those of the control group. There were no prominent histopathologic lesions in the kidneys of the pigs necropsied in the Cd diet treated group. The concentrations of Cd in the kidneys of the Cd diet treated group at the second slaughter (42.90 µg/g) may indicate that the levels obtained were not high enough to produce kidney damage and

therefore, the response to aflatoxin B_1 or warfarin was not affected by renal malfunctioning.

Blood Coagulation

Exposure to aflatoxin B_1 has produced prolongation of blood clotting times in the rat. Results of in vitro studies indicate that the anticoagulant effect is due mainly to competition with vitamin K in the production of prothrombin in the liver (Bassir and Bababunmi, 1972). Hemorrhage and significant prolongation in prothrombin time in swine consuming feed containing aflatoxin B_1 have been extensively described (Loosmore and Harding, 1961; Hauser et al., 1971; Neufville, 1974; Edds, 1979). In the same way, warfarin, an extensively used oral anticoagulant and rodenticide, functions as a vitamin K_1 antagonist (Fasco et al., 1977). Generally, poisoning occurs when animals ingest baits intended for rats and mice. Pigs are more susceptible to warfarin than are rats and mice. Swine, dogs and cats can be poisoned by eating rats and mice that were killed by these compounds (Hatch, 1977).

The only established function of vitamin K_1 and K_2 in man and other mammals is the synthesis of four plasma coagulation factors, i.e., II, VII, IX, X (Barkhan and Shearer, 1977; O'Reilly and Aggeler, 1970; Zieve and Solomon, 1969).

Attempts have been made to compare the blood anticoagulant properties of aflatoxin B_1 with those exhibited by 4-hydroxycoumarin, in view of the similarities in the structure of the synthetic coumarins and the aflatoxin (Asao et al., 1963; Bababunmi and Bassir, 1969).

Pigs in groups II (warfarin group) and VI (Cd + warfarin group) showed more rapid and higher increases, both in

prothrombin times (PT) and activated partial thromboplastin times (APPT) than those pigs in groups II (aflatoxin B_1 group) and V (Cd + aflatoxin B_1 group) during the first 6 days of the critical phase of the experiment (Figures 24 and 26). Values in the pigs receiving warfarin returned to normal after dosing had been suspended by the end of the experiment, while those in pigs with aflatoxin B_1 in group II increased significantly over the rest of the groups by the 10th day (Figures 24 and 26).

A marked difference can also be seen between the two warfarin treated groups (groups III and VI). Both PT and APTT were higher in group VI than in group III. Also, very significant differences were observed between the two aflatoxin B_1 groups (groups II and V). Both PT and APTT were higher in group II than in group V. Even more interesting was the fact that no significant increases in PT or APTT were observed in group V, where the mean values paralleled those of the control group (Figures 24 and 26).

Pigs were more susceptible initially with higher mean values in PT and APTT when dosed with warfarin than when dosed with aflatoxin B_1 (Figures 24 and 26). The difference in the responses to the anticoagulant properties of warfarin and aflatoxin B_1 could be associated with several factors that influence availability of the chemical at the site of action. These would include rate of intestinal absorption, rate of liver uptake and rate of liver biotransformation. The present experiment seems to support a difference in the bioactivation through the hepatic microsomal enzyme system

as demonstrated through the different parameters described in the result section (Figures 4, 16, 18, 20, 24 and 26).

It is not known whether warfarin must be activated by the liver to exert its anticoagulant properties. It has been demonstrated in rats that the mechanism of action of warfarin could be the direct inhibition of phylloquinone epoxide reductase, which prevents the normal regeneration of phylloquinone via vitamin K_1-epoxide cycle and causes phylloquinone 2,3 epoxide to accumulate in the liver (Matschiner et al., 1974; Ren, Laliberte and Bell, 1974; Sadowski and Suttie, 1974; Ren et al., 1977). On the other hand, it is known that aflatoxin B_1 requires metabolic activation to elicit its carcinogenic effects (Campbell and Hayes, 1976). The susceptibility of a species is directly correlated with the hepatic cytoplasmic reduction to aflatoxicol, but inversely related to its hydroxylated transformation to aflatoxin Q_1 or aqueous metabolites (Edds, 1979). It is also suggested that the active metabolite may be a 2,3 epoxide. A number of unsaturated compounds are readily epoxidized by the mixed function oxygenase of the liver microsomes (Garner et al., 1972). No correlation between phylloquinone 2,3 epoxide and the aflatoxin 2,3 epoxide metabolites have been made as far as toxicity is concerned.

Therefore, if aflatoxin B_1 requires metabolic activation to elicit its anticoagulant effects, this activation process could have affected the delayed effects of aflatoxin B_1 on PT and APTT, observed in Figures 24 and 26. When one compares

groups I (control group), II (aflatoxin B_1 group) and III (warfarin group).

There are wide species differences in hypoprothrombinemic response induced by oral coumarin related anticoagulants (Deckert, 1974; Hatch, 1977), as well as wide species differences in susceptibility to aflatoxin B_1 (Newberne et al., 1969; Edds, 1973) which may explain the disagreement of conclusions published by Bababunmi and Bassir (1969). Besides, the routes of administration and the types of coumarin related coumpounds used in each experiment were different.

After a period of 3 hours, aflatoxin B_1 had prolonged the normal clotting time maximmally in rats. The peak action of 4-hydroxycoumarin was reached after 48 hours in the same experiment (Babamunmi and Bassir, 1969). However, pigs are more susceptible to warfarin than are rats and mice. Ingestion of 0.05 - 0.4 mg/kg of body weight per day for 7 days can kill pigs. Rats and mice died after ingesting 1 mg/kg of body weight per day for 5 days (Clarke and Clarke, 1975; Buck et al., 1976; Hatch, 1977; Osweiler, 1978). Resistance to anticoagulant rodenticides by rats have been discovered (O'Reilly et al., 1964; Deckert, 1974). A very interesting extreme of this variability is represented by their own data where the amounts injected intraperitoneally were larger for 4-hydroxycoumarin (15.0 mg) than pure aflatoxin B_1 (17.5 µg). The intensity of the response was greater for 4-hydroxycoumarin (80.7 ± 0.2 seconds) than for aflatoxin B_1 (48.1 ± 0.1 seconds).

Cadmium induced differences in the intensity and duration of the respones in PT and APTT when pigs were dosed daily for 5 days with either aflatoxin B_1 or warfarin. Mean Cd concentrations in the livers of groups V and VI were 7.50 - 8.37 µg/g (Figure 37) respectively, in contrast to < 0.22 µg/g in livers of groups II and III at the time of the first slaughter. These differences were associated with the greater enzyme activities (AP, SDH, SGOT) observed in pigs in group II in contrast to group V (Figures 16, 18 and 20), as well as growth rate and the body weight suppression (Figure 4 and Table 4).

The different values in PT and APTT between groups II (aflatoxin B_1 group) and V (Cd + aflatoxin B_1 group) were not due to lack of intestinal absorption or liver uptake of aflatoxin B_1, since significant increase in mean values in SDH and SGOT (Figures 18 and 20) were observed in both groups. The differences in PT and APTT between groups III (warfarin group) and VI (Cd + warfarin group) were not due either to lack of intestinal absorption or liver uptake since it is known that warfarin intestinal absorption is complete (Clatanoff et al., 1954; Shapiro and Ciferri, 1957), as discussed earlier in this chapter. Besides, it was demonstrated that PT and APTT increased significantly in both groups when pigs were exposed to warfarin (Figures 24 and 26).

Kidney damage, which may influence the duration and intensity of response to oral anticoagulants (Deckert, 1974), and probably to aflatoxicosis too, was not involved in this instance, because of lack of changes in BUN, urinalysis or

histopathology. In general, there were no severe kidney toxic effects in any of the groups (except one animal in group II). Cd concentrations in the kidneys of the Cd diet treated group seemed to be too low (42.90 µg/g) to produce proteinuria. Therefore, one may conclude that the different responses in AP, SDH, SGOT, PT and APTT responses between pigs in groups II and V, III and VI were associated with the presence of Cd in the liver.

Publications on drug interactions with anticoagulants are probably more numerous than for other classes of drugs. Such interactions are common, usually therapeutically important and occasionally life-threatening, but they are also relatively easily detected and analyzed (Kock-Weser and Seller, 1971).

The interactions associated with drug biotransformations are of special interest. Two types of interactions are possible; stimulation or inhibition of the drug-metabolizing enzymes by other drugs or the anticoagulants (Deckert, 1974).

Aflatoxins may exert direct effects on nuclear DNA and RNA synthesis or indirectly through activation by the endoplasmic reticulum as described before (Hatch, 1977; Garner et al., 1972; Edds, 1979). The values of PT and APTT in groups II and V suggest that aflatoxin B_1 was activated by the liver microsomal enzyme system in order to produce an anticoagulant effect (Figures 24 and 26). The mean PT and the APTT values observed in pigs in group V (Cd + aflatoxin B_1) paralleled those of the group I (Control group). If the anticoagulant properties of aflatoxin B_1 had been elicited by a direct effect,

there would have been increased values in PT and APTT in group V, since significant high activities in serum enzymes (SDH and SGOT) were detected. Cd may have blocked the liver microsomal enzyme system and prevented the activation of aflatoxin B_1 to produce anticoagulant metabolites in group V. The greater effects of aflatoxicosis observed in group II, like the marked BW loss during and after dosing with aflatoxin B_1, as well as the high values in AP, SDH, SGOT, PT and APTT, are additional factors supporting this hypothesis.

Warfarin and dicoumarol are subject to the same major biotransformation pathways (Buck et al., 1976). Warfarin is hydroxylated by hepatic enzymes to inactive compounds, which are excreted in the urine (Buck et al., 1976; Goodman and Gilman, 1975). Therefore, if Cd blocked the microsomal enzyme system of the liver, an increase in intensity and duration in PT and APTT will be expected. In fact, this was observed in group VI (Cd + warfarin group), as demonstrated in figures 24 and 26. Two out of four pigs died in this group with subcutaneous hemorrhages and internal bleeding.

In summary, Cd may have blocked the liver microsomal enzyme system and prevented the activation of aflatoxin B_1 to produce anticoagulant effects in group V. On the other hand, Cd induced blockade prevented the inactivation of warfarin, enhancing its anticoagulant effects as demonstrated by the pigs in group VI.

The results and conclusions of this dissertation support the blocking effect of Cd on the hepatic microsomes reported

by others (Hadley et al., 1974; Teare et al., 1977). It is also feasible that the resulting effect of that blockade could be a potentiation or inhibition of other drug effects (Fouts and Pohl, 1971; Hadley et al., 1974; Teare et al., 1977).

This is the first suggestion of the Cd blocking effect on the microsomal enzyme system in pigs and by the oral route instead of by ip injection in mice or rats (Hadley et al., 1974; Teare et al., 1977) or in vitro inhibition (Hadley et al., 1974). The importance of this discovery could represent very valuable information for protection of human or animal health. In one way, it could be used to ameliorate the toxic effects of carcinogenic compounds, like aflatoxin B_1, by preventing its biotransformation to active carcinogenic metabolites. On the other hand, humans or animals exposed to subclinical levels of Cd when treated with oral anticoagulants or accidentally poisoned with rodenticides, such as warfarin, may result in a life-threatening situation (Koch-Weser and Seller, 1971).

It was also demonstrated that aflatoxin B_1 may possess the same property of the anticoagulant rodenticides, that is, being more toxic when ingested daily over a period of 5 - 7 days. The only necessity is that animals must be free of liver microsomal enzyme blockade induced by agents such as Cd, so the biotransformation of aflatoxin B_1 to an active metabolite can occur. This principle is associated mainly with the anticoagulant effects of aflatoxin B_1, but probably can be extended to its hepatotoxic properties, too. The work done by Cysewski et al. (1968) utilized 1.98 mg/kg of aflatoxin B_1 as a single dose to reach toxicity in young pigs of 11.4 -

21.4 kg average body weight. In the present trial, 5 daily oral doses of 0.2 mg of aflatoxin B_1/ kg body weight in young pigs of 16.0 - 32.4 kg of body weight, were proven to be toxic.

The maximum peak values in PT and APTT obtained in group II were 6 days after the maximum peak concentration of AP in the same group (Figures 16, 24 and 26). One may hypothesize that a lack of bile flow could diminish the intestinal absorption of vitamin K_1 enhancing the toxicity of aflatoxin B_1 as demonstrated by the reduction of PT when vitamin K_1 was provided to pigs (Hauser et al., 1971; Neufville, 1974).

The displacement of warfarin from plasma binding sites induced by Cd itself, or through the metallothionein formation, were not measured in this experiment. If this displacement happens to occur, warfarin toxicity will result with the combination of hepatic microsomal enzyme system inhibition. However, the displacement of warfarin was less likely to occur, since the sojourn of Cd in blood is brief and its concentration in blood is extremely low (Petering et al., 1973). Besides, if displacement of warfarin had occurred in group VI, a similar effect would have been detected in group V with high values in PT. As discussed before, the mean PT values of pigs in group V paralleled those of group I (control group).

A significant decrease in concentration of F ($P < 0.0279$) was determined in group II at the 10th day of the critical phase of the treatment (Figure 28). Liver is the sole source of fibrinogen and prothrombin. Liver damage induced by aflatoxin

B_1 and its characteristic of protein synthesis inhibition could have contributed to the lowering values of F observed at the 10th day of the critical phase of this experiment as suggested by Osuna et al., (1977).

Serum Proteins

A number of investigations have been directed toward determining the effect of aflatoxin on the antibody response and the serum proteins in animals (Annau et al., 1964; Gumbmann and Williams, 1969; Edds, 1973; Osuna et al., 1977). The rationale of these investigations was that aflatoxin B_1 inhibits protein synthesis and, therefore, could inhibit antibody formation. Serum changes occur in aflatoxicosis mainly in alpha and beta globulin levels which were depressed, and in the gamma globulin levels which were increased (Annau et al., 1964; Osuna et al., 1977).

Mice exposed to subclinical doses of cadmium chloride (3 µg/g) for 10 weeks and inoculated with antigen 6 weeks after discontinuance of exposure had a remarkable decrease in antibody-forming cells, particularly IgG. These results indicate that the immunosuppression continues and may be more pronounced several weeks after exposure to Cd than during administration of the metal (Koller et al., 1975).

Plasma protein levels can be used as a test for liver function (Coles, 1967; Tietz, 1976). The proteins such as albumin, fibrinogen and the majority of the globulins, with the exception of the gamma globulins, are synthesized by the liver (Tietz, 1976).

The electrophoretic and chemical studies performed on the serum samples of young pigs confirmed that those animals exposed to aflatoxin B_1 alone in group II were the most affected with severe liver damage. The highly toxic state present in pigs in group II (aflatoxin B_1 group) at the end of the critical phase of the experiment was indicated by significantly altered values in TP ($P < 0.0378$), αG ($P < 0.0133$), βG ($P < 0.00119$), γG ($P < 0.05$), as well as F ($P < 0.0279$), as discussed before (Figures 28, 30, 32, 33 and 34).

One may suggest that the pigs in group II, under the circumstances described above, were very sick. Animals in this group were losing weight during and after dosing with aflatoxin B_1 (Figure 4 and Table 4), along with loss of apetite. Many experiments have indicated a direct relationship between the quantity and quality of ingested protein and the formation of plasma proteins, including antibody formation (Osuna et al., 1977). Thus, animals without appetite, losing weight and with a poor protein profile, have reduced changes of recovery. Under these conditions, secondary infections or parasitic diseases may appear, complicating the clinical picture.

Cd induced low values of gamma globulin, as demonstrated in group IV (Figure 34) at the 6th day ($P < 0.0198$) of the critical phase of the experiment. One animal died in this group with signs of pneumonia and anemia. It was also reported earlier that the Cd diet treated pigs presented lowered WBC counts through the first 4 weeks of the experiment. One may

hypothesize that pigs exposed to Cd may be predisposed to secondary infections or parasitic diseases due to immunosuppression (Koller et al., 1975) and/or low WBC counts.

Metal Residues

Cd is of interest in animal agriculture because of its toxicity; it is not an essential element (Neathery and Miller, 1976). Practices such as use of superphosphate fertilizer and municipal sewage sludge on farmland can make Cd available in the livestock-human foodchains.

Cd was found to accumulate steadily in both kidney and liver in pigs treated with 83 µg/g Cd diet from the first to the second slaughter period (Figures 35 and 36), as reported by others (Schroeder, 1967; Piscator and Lind, 1972). Highest Cd concentrations were present in the kidneys, followed by liver tissues; this is in agreement with previous reports (Friberg et al., 1971; Neathery and Miller, 1975). Urine Cd concentrations were not well correlated with tissue concentrations either (Carlson and Friberg, 1957; Petering et al., 1973). Cd was found to interfere with the metabolism of Fe, Zn and Cu, as demonstrated before (Six and Goyer, 1972; Stonard and Webb, 1976). Finally, animal muscle residue levels of the Cd treated pigs were very low and probably would pose no hazard for human health (Neathery and Miller, 1976; Osuna et al., 1979).

A loss of iron from the liver and kidney tissues was observed in the 83 µg/g Cd diet treated group, especially loss of iron from the liver tissue (Figures 38 and 39). Others

have found that the overall effect of Cd is to lower liver iron (Bunn and Matrone, 1966; Stonard and Webb, 1976; Osuna et al., 1979). Accumulation of Cd was associated with an increased zinc concentration in the kidney (Figures 41 and 42) and low concentration in the liver tissues. Similar results in tissue Zn concentrations were reported when young pigs were exposed daily to 83 μg/g Cd diet for 9 weeks (Osuna et al., 1979). Exposure of pigs to a Cd diet resulted in an increased Cu concentration in the kidney tissues (Figures 44 and 45), as reported by others (Stonard and Webb, 1976; Osuna et al., 1979). There was no effect on calcium tissue concentrations (Figures 47 and 48) induced by Cd accumulation.

Although inhibition by Cd of iron metabolism is due primarily to direct competition between these cations for a mucosal binding site other than thionein (Hamilton and Valberg, 1974), an indirect response, i.e., secondary to the primary interactions between Cd and Cu or Zn, as suggested initially (Hill et al., 1963) , also remains possible (Stonard and Webb, 1976).

The iron deficiency anemia is supposed to be produced mainly by inhibition of iron absorption (Freeland and Cousins, 1973 ; Prigge et al., 1977). Therefore, the microcytic hypochromic anemias are specific for iron deficiency or failure to utilize iron. Copper and pyridoxine deficiencies, as well as chronic blood loss have been described as cause of failure to utilize iron.

The animal defense mechanisms against iron deficiency anemia seems to be active even in the presence of Cd diets, as demonstrated by the lowered Fe excretion through the urine in the 83 µg/g Cd group (Figures 38 and 39) during the first ($P < 0.0240$) and second ($P < 0.0131$) slaughter, as well as by increasing the protection (intestinal absorption) offered by oral iron administration against Cd induced anemia in swine (Pond et al., 1973).

Urine concentrations of Zn and Cu (Figures 41, 42, 44 and 45) did not correlate well with the kidney tissue concentrations of Zn and Cu respectively. It is possible that the high values of Zn and Cu found in the kidney tissues were an indication of their excretion through the urine, as a result of Cd induced the displacement of these elements from other tissues. Probably, the high values of Zn and Cu in the kidney tissues were the result of metallothioneins formation as the organism reacted to the presence of Cd.

Pulido et al., (1966) have shown that human kidney metallothionein has a molecular weight of 10,500 and that this protein contains 2.6 percent Zn, 4.2 percent Cd, 0.5 percent Hg and 0.3 percent Cu by weight. A metal-binding protein, metallothionein, has also been isolated from horses by Kägi and Vallee (1961). Since then, several investigators have described the phenomenon in chickens (Weser et al., 1973), mice (Nordberg et al., 1971), rats (Weser et al., 1973; Chen et al., 1975) and rabbits (Nordberg et al., 1972).

The demonstration that liver and kidney of the horse contain metallothioneins of similar composition, but of different ratios of Zn to Cd, which indicates, in fact, that the metal selectivity of these proteins is determined primarily by physiological circumstances rather than by their polypeptide structure (Kojima and Kägi, 1978). One may suggest that liver and kidney metallothionein of swine may contain different ratios of Zn to Cd, as indicated by higher Zn concentration in the kidney and lower Zn concentration in the liver of the 83 µg/g Cd diet pigs than corresponding values of the control group (Figures 41 and 42). It is also possible to expect different composition of elements in the metallothioneins of swine and, as has been demonstrated, there are differences between those of the humans and horses (Pulido et al., 1966; Kägi and Vallee, 1961).

Copper and/or zinc-thioneins also occur normally in variable amounts in the liver, kidneys and intestines of different mammalian species and, at least in the livers of sheep and calves, the total content of these thionein-bound cations seems to depend upon the zinc-status of the animal (Stonard and Webb, 1976).

It has been suggested that the intestinal copper- and zinc-thioneins function in the transport and/or absorption of Cu and Zn, the competition between Cd and these cations for the cation-binding-SH group of thionein being responsible for the antagonism by the former of the absorption of the latter (Evans et al., 1970). Thus, in addition to any changes in organ distribution,

the uptake of Cu and Zn also may be inhibited through the formation of Cd-thionein in the intestinal mucosa (Stonard and Webb, 1976).

Zinc muscle concentrations in pigs in group VI, at the time of the second slaughter, was found to be high (Figure 43). The muscle histopathology of these groups revealed a variation in size and shape of bundles of muscle fibers.

Cd absorption may be increased with low protein or low calcium diets with certain diseases or when fecal elimination is blocked (Neathery and Miller, 1975). Response to Cd is affected by health, nutrition, age, sex, stress and pattern of exposure (Webb, 1975).

Proteins play an important role in the formation of metallothioneins and other cation-thioneins (Zn-thionein, Cu-thionein), as well as in the blood transport of minerals (besides other essential functions) as demonstrated by Shaikh and Lucis (1972) with the electrophoretic separation of rat plasma. There is also a direct relationship between the quality of ingested protein and the formation of plasma protein, including antibody formation. It is known that Cd induces loss of appetite (Suzuki and Yoshida, 1978) and immunosuppression (Koller et al., 1975).

Therefore, the lack of good quality and quantity of protein and the aggravating factor, the loss of appetite, may increase the susceptibility of animals to Cd toxicity by two possible mechanisms:

1. Decreasing the animals' capabilities to bind the toxic Cd ion through the diminishing formation of metallothioneins.

2. Increasing susceptibility of animals to secondary diseases by enhancing the immunosuppression induced by Cd.

Pathology

The pathologic examination of kidney, liver and muscle tissues collected at the first slaughter from the control and cadmium exposed groups of pigs, prior to dosing with aflatoxin B_1 or warfarin, revealed minor, non-specific lesions. The condition of the pigs in the 2 groups were very similar.

The pathologic examination of the kidney, liver and muscle tissues collected at the second slaughter, following dosing of the pigs with aflatoxin B_1 or warfarin, while exposed to two Cd diet levels (0 and 83 µg/g Cd diet) revealed severe lesions in group II (aflatoxin B_1 group). The gross pathology indicated that pigs in groups II and VI were the most affected.

It was confirmed that the liver was the most affected organ following the administration of aflatoxin B_1 in young growing pigs. The most extensive lesion described was the severe fatty degeneration and vacuolation through every lobule.

Fatty degeneration is synonymous with fatty infiltration. Cellular infiltrations are a form of cell degeneration and characterized by an excess intracellular accumulation of various metabolic materials. Infiltration may occur as the result of genetic enzymatic deficiencies, or may also occur when cellular metabolism is disrupted (Smith et al., 1972).

Fatty infiltration is an excessive intracellular accumulation of lipids, primarily in the form of triglycerides and free fatty acids. Many toxins producing fatty liver cause protein synthesis inhibition. Aflatoxin B_1 impairs protein synthesis by inhibiting the activity of the DNA and RNA polymerases (Edds, 1973; Buck et al., 1976; Wogan and Shank, 1971). Aflatoxin B_1 may block protein synthesis at the polyribosomal level and, thereby, prevents aproprotein formation required for lipoprotein formation, a mechanism described for carbon tetrachloride and puromycin (Smith et al., 1972). The tryglycerides can not be mobilized out of the cell without lipoprotein formation and fatty infiltration results.

Pigs in group V (Cd + aflatoxin B_1 group) were protected by Cd against fatty infiltration induced by the aflatoxin B_1 supplement in group II. On the contrary, livers of pigs in group V had undergone hydropic degeneration, characterized by intracellular accumulation of fluids. However, hydropic degeneration is frequently an acute response to injury in any cell (Smith et al., 1972). The clinical chemistry analyses for SDH and SGOT (Figures 18 and 20) and the hydropic degeneration found in the livers of group V, collected at the time of the second slaughter of animals in this experiment, are excellent indicators that aflatoxin B_1 was absorbed from the gastrointestinal tract and affected the livers. However, the minor lesions observed in the livers of pigs from group V, compared to the severe fatty infiltration described in those from group II, can be considered as a protective action induced

by Cd, probably by preventing the biotransformation of aflatoxin B_1 into active toxic metabolites. Also, the good correlation between the clinical chemistry and the histopathology found in this group V can be used to emphasize that the different values in PT and APTT between groups II and V were not due to lack of intestinal absorption or liver uptake of aflatoxin B_1.

An unexpected finding in group VI (Cd + warfarin group) was the variation in size and shape of bundles of muscle fibers. There is not a direct explanation for it. The changes were probably induced by the severe intramuscular hemorrhage and lameness. It was found that the Zn concentration in the muscle of pigs of this group was much higher (Figure 43) than in the other groups.

Two animals died suddenly in group VI. The thoracic cavity of one animal was blood-filled and the other one presented severe subcutaneous hemorrhages in the ventral surface of the neck. The gross and histopathologic lesions described in group VI may indicate an additive toxic effect of warfarin when Cd is present in the diets of young pigs.

One may suggest that, in bleeding disorders affecting the pigs, it is possible to distinguish between aflatoxicosis and warfarin toxicosis. Several techniques can be used for differential and accurate diagnosis, including clinical chemistry analyses (SDH, AP, SGOT, PT, APTT), the histopathology of the liver, dosage with vitamin K_1 with relief in warfarin toxicosis, but less in aflatoxicosis and the determination of aflatoxin B_1 or warfarin in feed or tissues.

No effects were produced in the control group as induced by the presence of aflatoxin B_1 in the feed at < 20 ng/g. Florida's humidity and temperature levels are favorable for the presence and development of aflatoxin B_1 in feed. The 20 ng/g of aflatoxin B_1 is the guideline level established by U.S.D.A. and U.S. F.D.A. for feeds and foods intended for interstate shipment and for animal consumption (Edds, 1979). This level is accepted as below the toxic level for animals and would not result in detectable levels of aflatoxin B_1 or its metabolites in tissues intended for human consumption.

No effects were induced by pathogens or leptospirosis, as no bacterial pathogens were isolated from the 6 groups of experimental animals. Leptospiral titers remained negative. In the same way, the pigs were free of parasitic ova and, therefore, no effects were expected.

The feeds were found free of organochlorides, organophosphates and carbamates.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Thirty-six healthy, weaned barrows, mixed breed, averaging 9 kg of body weight, were assigned at random to 6 treatment groups, 6 pigs per group: Group I - negative control; Group II - 0.2 mg/kg of aflatoxin B_1; Group III - 0.2 mg/kg of warfarin; Group IV - 83 µg/g of cadmium diet (given as cadmium chloride); Group V - 83 µg/g of cadmium diet (given as cadmium chloride) plus 0.2 mg/kg of aflatoxin B_1; Group VI - 83 µg/g of cadmium diet (given as cadmium chloride) plus 0.2 mg/kg of warfarin. Groups II, III, V and VI received 5 daily doses of the chemical. The experiment was designed to compare 2 levels of cadmium (0.0 and 83 µg/g Cd diet) in three groups of pigs, i.e., control, aflatoxin B_1 and warfarin.

Cadmium (Cd) was provided daily through the diets during the 40 days of the experiment. Aflatoxin B_1 and warfarin were given daily per os in a gelatin capsule for five days during the 5th week of the experiment and the effects were followed for 10 days during the critical phase of the experiment.

Three years of acute and chronic toxicity studies of the "Health Effects of Sewage Sludge in Animals," sponsored by the Environmental Protection Agency, has demonstrated that cadmium may be concentrated in urban sewage sludge. One lot

of sewage sludge from Chicago contained high levels of Cd (165 µg/g). A previous trial compared the effects of feeding 3 groups of weanling pigs a starter ration, the starter ration containing 50 percent of this dried, activated, Chicago sewage sludge vs. the starter ration, 18 percent standard crude protein basal diet, containing 83 µg/g Cd for 9 weeks (Osuna et al., 1979.

The purpose of again using 83 µg/g cadmium diet in pigs was to determine whether animals may be predisposed to higher risk when exposed to moldy feed containing aflatoxin B_1 or other poisons, such as rodenticides, even if no histopathological liver lesions are detectable in pigs consuming the Cd diet.

The objectives of this experiment were to compare the toxic effects of aflatoxin B_1, a dihydrofuranocoumarin, and warfarin, a 3-(α-acetonyl-benzyl)-4 hydroxycoumarin; and also to determine whether an additive effect from either aflatoxin B_1 or warfarin occurs when cadmium is present in higher than normal levels in the diets of young pigs.

The body weight loss ($P < 0.0065$) in the aflatoxin B_1 group was associated with decreased feed consumption, decreased protein synthesis and liver damage (Edds, 1979; Cysewski et al., 1978; Osuna et al., 1977). Low significant values in serum protein ($P < 0.0378$), αG ($P < 0.0133$), βG ($P < 0.00119$), γG ($P < 0.05$) and fibrinogen ($P < 0.0279$) were induced by aflatoxin B_1.

High values of red blood cell ($P < 0.05$), packed cell volume ($P < 0.0104$), hemoglobin ($P < 0.0188$) were associated with dosing of aflatoxin at the 6th day of the critical phase of the treatment. However, this stage was followed by abrupt drops in RBC, PCV and Hb values and highly significant increases in prothrombin time (PT) ($P < 0.01$) and activated partial thromboplastin time (APTT) ($P < 0.038$) at the 10th day of the final phase of the experiment.

The significantly increased values of alkaline phosphatase (AP) ($P < 0.016$), sorbitol dehydrogenase (SDH) ($P < 0.003$), aspartate aminotransferase (SGOT) ($P < 0.05$) were determined 48 hours after initiation of the dosing with aflatoxin B_1. The hepatotoxic effects of aflatoxin B_1 were also correlated with hepatic fatty infiltration and vacuolation through each and every lobule in 3 out of 4 pigs in the group. It was also demonstrated that aflatoxin B_1 is very toxic when ingested daily over a period of 5 days, a property described for warfarin (Osweiler, 1978; Hatch, 1977).

Warfarin was more effective in producing earlier and higher significantly increased values in PT ($P < 0.0001$) and APTT ($P < 0.007$) than those receiving aflatoxin B_1 by the second day after initiation of dosage. However, these values in the pigs receiving warfarin returned to normal after dosing had been suspended at the end of the experiment, while those in the pigs of aflatoxin B_1 group increased significantly ($P < 0.01$ and $P < 0.038$ respectively) by the 10th day of the critical phase of the experiment. No liver or kidney damage was induced by warfarin.

Depressed growth, feed consumption and feed efficiency were evident in the pigs consuming 83 µg/g Cd in the diet. Growth retardation induced by dietary Cd is mainly due to reduced feed consumption (Suzuki and Yoshida, 1978). Probably, the free Cd displaces essential cations from enzymes and nucleic acids affecting their physiological function and causing toxicity (Suzuki and Yoshida, 1978; Vallee and Ulmer, 1972; Shaikh and Smith, 1976).

An extreme microcytic hypochromic anemia was observed in young growing pigs fed 83 µg/g Cd diet ($P < 0.0001$) by the 4th week of the experiment. A loss of iron from the liver ($P < 0.0001$) and kidneys ($P < 0.0001$) was also observed in the dietary Cd treated pigs.

Highest Cd concentrations were determined in the kidney ($P < 0.0001$), followed by liver ($P < 0.0001$) as reported by others (Schroeder, 1967; Piscator and Lind, 1972). Urine Cd concentration was not well correlated to kidney tissue concentration (Carlson and Friberg, 1957; Petering et al., 1973), suggesting that the high Cd values in the kidney are not an indication of their excretion through the urine but the result of metallothionein formation as the body reacts to the presence of Cd (Kägi and Vallee, 1961; Pulido et al., 1966; Nordberg et al., 1971 and 1972; Weser et al., 1973; Chen et al., 1975).

Cadmium was also found to interfere with the metabolism of iron, zinc and copper, as demonstrated earlier (Six and Goyer, 1972; Stonard and Webb, 1976). Urine iron (Fe), zinc (Zn) and copper (Cu) concentrations again did not correlate well with kidney concentrations of Fe, Zn and Cu. Pulido et al., (1966) had demonstrated that Zn and Cu are incorporated in the formation of human kidney metallothionein. Stonard and Webb (1976) stated that copper and/or zinc-thioneins also occur in variable amounts in the liver, kidney and intestines of different mammalian species.

Finally, animal muscle residue values of the Cd treated pigs were very low (< 0.22 μg/g) and probably would pose no immediate hazard for human health under normal conditions of swine management (Neathery and Miller, 1976; Osuna et al., 1979).

Lowering WBC counts ($P < 0.0056$) and reducing gamma globulin values ($P < 0.0118$) in the Cd diet treated group may predispose animals to secondary infections or parasitic diseases due to immunosuppression and may explain the death and pneumonia of one pig in the Cd diet treated group.

Cadmium prevented pigs against the fatty hepatocytic infiltration but not hydropic degeneration induced by aflatoxin B_1. Cadmium induced differences in the intensity and duration of response in prothrombin time (PT) and activated partial thromboplastin time (APTT) when pigs were dosed daily for 5 days with aflatoxin B_1 or warfarin. The PT and APTT levels of the Cd diet treated pigs dosed with aflatoxin B_1 paralleled the

values of the control group with no significant changes. On the other hand, the PT and APTT of the Cd diet treated pigs dosed with warfarin increased more rapidly than those dosed with warfarin alone and were highly significant ($P < 0.0008$, $P < 0.008$) over those values of the control group.

Cadmium also induced differences in the activity of serum enzymes (AP, SDH, SGOT) when pigs were exposed to aflatoxin B_1 or warfarin. In the same way, Cd prevented greater body weight losses and decreased feed consumption when dosed with aflatoxin B_1 than those exposed to aflatoxin B_1 alone. However, Cd increased the toxicity of warfarin with severe lameness and subcutaneous hematomas in the ventral surface of the jaws and neck, decreasing the feed consumption and rate of growth.

Cadmium may have blocked the liver microsomal enzyme system and prevented the activation of aflatoxin B_1 to a toxic anticoagulant metabolite. On the other hand, that blockade may have prevented the inactivation of warfarin, enhancing its anticoagulant effects. It is concluded that there is an inhibitory effect on aflatoxin B_1, while there is an enhancing synergistic effect with warfarin when Cd is present in the diets of young pigs at 83 µg/g. This Cd diet concentration resulted in 35.13 µg/g and 6.80 µg/g Cd tissue accumulation in kidney and liver respectively at the fourth week of the experiment. No prominent lesions were found in the kidney or liver at these concentrations.

This is the first suggestion of Cd blocking effect on the microsomal enzyme system performed on pigs and by the oral

route instead of by ip injection in mice or rats (Hadley et al., 1974; Teare et al., 1977) or in vitro (Hadley et al., 1974). This blockade was demonstrated through clinical signs, clinical chemistry, as well as gross and histopathologic lesions. The levels of Cd in tissue or feed add support to these findings.

APPENDIX

Table 5. Values of body weight (kg) in young pigs (see Figure 3).

Group	N*	Week	Mean	Standard Deviation	Range
Control	18	0	9.07	2.74	5.4 - 15.1
	18	1	10.74	3.44	5.6 - 16.0
	18	2	13.90	4.40	6.6 - 21.8
	18	3	17.56	5.75	7.8 - 26.8
	12	4	22.23	4.93	16.0 - 32.4
83 µg/g Cd	18	0	9.01	2.82	5.6 - 15.6
	18	1	10.70	3.22	6.2 - 19.0
	18	2	12.90	3.82	7.8 - 22.8
	18	3	16.03	4.82	9.2 - 27.6
	11	4	18.65	3.57	15.2 - 24.8

*N = Number of observations.

Table 6. Values of body weight (kg) in young pigs (See Figure 4).

Group	N*	Day	Mean	Standard Deviation	Range
I	4	0	22.10	2.43	19.8 - 25.0
	4	10	28.40	2.50	26.0 - 31.2
II	4	0	22.30	7.31	16.0 - 32.4
	4	10	18.95	7.34	12.6 - 27.4
III	4	0	22.30	5.46	17.6 - 29.8
	4	10	28.25	5.20	23.6 - 35.2
IV	3	0	19.66	4.83	15.2 - 19.0
	3	10	26.20	5.85	19.8 - 27.8
V	4	0	17.20	3.65	12.6 - 21.4
	4	10	16.70	4.56	12.2 - 22.6
VI	4	0	19.35	3.02	16.0 - 23.0
	4	10	21.50	4.46	17.0 - 27.6

*N = Number of observations.

Table 7. Values of mean corpuscular volume (fl) in young pigs (See Figure 5).

Group	N*	Week	Mean	Standard Deviation	Range
Control	18	0	52.52	4.23	42.0 - 59.5
	18	1	51.05	3.36	41.5 - 57.0
	18	2	53.02	3.60	46.0 - 58.0
	18	3	52.83	3.24	47.0 - 58.5
	12	4	54.08	2.66	50.0 - 57.5
83 µg/g Cd	18	0	52.69	3.53	47.5 - 63.0
	18	1	49.69	2.53	45.0 - 53.0
	18	2	48.94	2.97	42.5 - 53.0
	18	3	46.11	3.39	40.0 - 53.0
	11	4	42.95	3.70	38.0 - 51.0

*N = Number of observations.

Table 8. Values of mean corpuscular volume (fl)* in young pigs (See Figure 6).

Group	N**	Day	Mean	Standard Deviation	Range
I	4	0	55.37	1.25	54.0 - 57.0
	4	2	58.00	1.58	56.5 - 60.0
	4	4	58.12	1.49	56.5 - 60.0
	4	6	57.62	1.25	56.0 - 59.0
	4	10	57.00	1.08	55.5 - 58.0
II	4	0	53.50	3.39	50.0 - 57.5
	4	2	56.50	3.10	53.0 - 60.0
	4	4	57.12	1.93	54.5 - 59.0
	4	6	56.25	2.01	53.5 - 58.5
	4	10	53.37	2.69	53.5 - 56.5
III	4	0	53.57	3.09	50.0 - 56.0
	4	2	55.12	2.92	51.5 - 57.5
	4	4	55.75	4.17	51.0 - 59.5
	4	6	55.50	3.89	51.0 - 59.0
	4	10	53.87	3.77	49.5 - 57.5
IV	3	0	42.33	1.75	40.5 - 44.0
	3	2	42.83	2.51	40.0 - 45.5
	3	4	41.16	2.02	39.0 - 43.0
	3	6	40.00	0.86	39.0 - 40.5
	3	10	37.00	1.32	36.0 - 38.5

Table 8 - continued

Group	N*	Day	Mean	Standard Deviation	Range
V	4	0	45.62	4.71	40.0 - 51.0
	4	2	47.62	6.04	40.0 - 54.0
	4	4	47.50	7.15	38.5 - 55.0
	4	6	46.25	6.34	38.5 - 53.5
	4	10	42.75	7.36	34.0 - 51.0
VI	4	0	40.75	2.25	40.5 - 43.5
	4	2	42.12	1.75	40.0 - 44.0
	4	4	40.62	2.59	37.0 - 42.5
	4	6	39.37	2.01	37.0 - 41.5
	2	10	36.50	1.41	35.5 - 37.5

*f1 = p3

**N = Number of observations.

Table 9. Values of hemoglobin (gm%) in young pigs (See Figure 7).

Group	N*	Week	Mean	Standard Deviation	Range
Control	18	0	13.87	1.00	11.8 - 15.2
	18	1	12.52	1.18	10.2 - 13.7
	18	2	12.27	1.05	10.2 - 13.8
	18	3	12.27	1.29	10.1 - 13.7
	12	4	12.49	1.46	9.4 - 14.2
83 µg/g Cd	18	0	13.81	1.36	11.3 - 16.2
	18	1	12.06	1.11	10.4 - 14.5
	18	2	11.41	0.96	9.5 - 13.4
	18	3	10.22	1.22	8.1 - 13.4
	11	4	9.20	1.10	7.1 - 11.1

N = Number of observations.

Table 10. Values of hemoglobin (gm%) in young pigs (See Figure 9).

Group	N*	Day	Mean	Standard Deviation	Range
I	4	0	12.17	1.07	11.0 - 13.4
	4	2	12.30	1.00	11.3 - 13.7
	4	4	12.22	0.82	11.3 - 13.3
	4	6	12.20	0.99	11.2 - 13.5
	4	10	12.02	0.49	11.4 - 12.6
II	4	0	12.05	1.97	9.4 - 14.1
	4	2	13.57	2.05	10.6 - 15.3
	4	4	15.05	1.84	12.3 - 16.0
	4	6	15.35	3.44	11.1 - 18.4
	4	10	10.85	6.51	3.7 - 17.0
III	4	0	13.25	1.27	11.4 - 14.2
	4	2	12.62	1.05	11.2 - 13.7
	4	4	12.87	1.18	11.1 - 13.6
	4	6	13.45	1.51	11.3 - 14.8
	4	10	13.12	1.04	12.7 - 14.3
IV	3	0	8.86	0.23	8.6 - 9.0
	3	2	8.83	1.10	8.3 - 10.1
	3	4	8.46	0.77	7.6 - 9.1
	3	6	8.03	0.83	7.5 - 9.0
	3	10	7.63	0.95	6.7 - 8.6

Table 10 - continued

Group	N*	Day	Mean	Standard Deviation	Range
V	4	0	9.72	1.82	7.1 - 11.1
	4	2	10.07	2.86	6.2 - 13.0
	4	4	10.17	2.76	6.3 - 12.4
	4	6	11.27	3.06	6.9 - 13.6
	4	10	11.70	3.71	6.7 - 15.2
VI	4	0	8.95	0.36	8.5 - 9.3
	4	2	8.20	0.18	8.0 - 8.4
	4	4	7.85	0.40	7.5 - 8.4
	4	6	7.75	0.95	6.7 - 9.0
	2	10	7.65	0.35	7.4 - 7.9

*N = Number of observations.

Table 11. Values of packed cell volume (%) in young pigs (See Figure 9).

Group	N*	Week	Mean	Standard Deviation	Range
Control	18	0	39.72	3.25	35 - 49
	18	1	35.00	3.28	30 - 42
	18	2	34.55	2.89	30 - 40
	18	3	35.33	3.46	29 - 40
	12	4	36.33	4.11	32 - 43
83 µg/g Cd	18	0	39.61	3.88	32 - 48
	18	1	34.16	3.61	30 - 43
	18	2	32.94	2.33	28 - 37
	18	3	29.77	2.51	25 - 34
	11	4	28.00	3.22	21 - 32

*N = Number of observations.

Table 12. Values of packed cell volume (%) in young pigs (See Figure 10).

Group	N*	Day	Mean	Standard Deviation	Range
I	4	0	35.75	3.50	32 - 40
	4	2	37.25	3.30	35 - 42
	4	4	36.00	2.16	34 - 39
	4	6	35.25	1.25	34 - 37
	4	10	36.50	1.73	35 - 38
II	4	0	35.73	6.18	28 - 43
	4	2	41.50	5.06	34 - 45
	4	4	43.50	5.91	35 - 48
	4	6	46.00	10.48	33 - 55
	4	10	31.25	18.19	11 - 49
III	4	0	37.50	3.00	34 - 40
	4	2	37.75	2.87	34 - 41
	4	4	37.25	2.87	33 - 39
	4	6	39.00	5.35	34 - 45
	4	10	38.00	2.16	35 - 40
IV	3	0	28.00	2.00	26 - 30
	3	2	28.33	3.21	26 - 32
	3	4	26.66	1.52	25 - 28
	3	6	25.66	3.21	22 - 28
	3	10	25.00	2.64	23 - 28

Table 12 - continued

Group	N*	Day	Mean	Standard Deviation	Range
V	4	0	28.75	5.25	21 - 32
	4	2	30.00	6.87	20 - 35
	4	4	31.75	7.80	21 - 38
	4	6	34.50	9.57	21 - 43
	4	10	36.00	6.37	27 - 41
VI	4	0	27.25	1.70	25 - 29
	4	2	26.25	0.95	25 - 27
	4	4	24.50	1.00	23 - 25
	4	6	21.75	1.70	20 - 24
	2	10	25.00	0.00	25 - 25

*N = Number of observations.

Table 13. Values of red blood cell counts (millions/µl) in young pigs (See Figure 11).

Group	N*	Week	Mean	Standard Deviation	Range
Control	18	0	7.60	0.92	6.10 - 9.08
	18	1	6.76	0.89	5.42 - 8.77
	18	2	6.47	0.18	5.33 - 7.77
	18	3	6.68	0.80	5.57 - 8.41
	12	4	6.72	0.91	5.05 - 8.30
83 µg/g Cd	18	0	7.51	0.69	6.64 - 9.00
	18	1	6.81	0.81	5.93 - 8.45
	18	2	6.68	0.53	5.84 - 7.93
	18	3	6.43	0.50	5.53 - 7.23
	11	4	6.50	0.52	5.41 - 7.14

*N = Number of observations.

Table 14. Values of red blood cell (millions/µl) in young pigs (See Figure 12).

Group	N*	Day	Mean	Standard Deviation	Range
I	4	0	6.41	0.71	5.71 - 7.31
	4	2	6.38	0.74	5.84 - 7.43
	4	4	6.10	0.51	5.62 - 6.79
	4	6	6.26	0.50	5.75 - 6.73
	4	10	6.27	0.42	5.89 - 6.79
II	4	0	6.70	1.42	5.05 - 8.30
	4	2	7.27	1.15	5.76 - 8.18
	4	4	7.56	1.14	6.05 - 8.69
	4	6	8.10	1.50	5.99 - 9.53
	4	10	5.79	3.20	2.09 - 9.10
III	4	0	7.06	0.46	6.47 - 7.61
	4	2	6.83	0.48	6.30 - 7.43
	4	4	6.57	0.61	6.03 - 7.45
	4	6	7.00	0.53	6.41 - 7.60
	4	10	7.04	0.54	6.65 - 7.84
IV	3	0	6.61	0.36	6.20 - 6.90
	3	2	6.69	0.38	6.26 - 7.01
	3	4	6.38	0.13	6.22 - 6.47
	3	6	6.25	0.33	5.87 - 6.50
	3	10	6.72	0.41	6.37 - 7.18

Table 14 - continued

Group	N*	Day	Mean	Standard Deviation	Range
V	4	0	6.24	0.60	5.41 - 6.85
	4	2	6.18	0.78	5.02 - 6.70
	4	4	6.58	0.79	5.40 - 7.08
	4	6	7.36	0.91	6.12 - 8.34
	4	10	8.05	1.07	6.68 - 8.95
VI	4	0	6.67	0.55	6.03 - 7.14
	4	2	6.30	0.41	5.82 - 6.77
	4	4	6.08	0.49	5.55 - 6.68
	4	6	5.92	0.90	4.81 - 6.95
	2	10	6.86	0.14	6.76 - 6.96

*N = Number of observations.

Table 15. Values of white blood cell (thousands/µl) in young pigs (See Figure 13).

Group	N*	Week	Mean	Standard Deviation	Range
Control	18	0	20.3	3.5	15.1 - 26.7
	18	1	22.2	3.8	14.2 - 29.7
	18	2	21.3	4.5	12.5 - 29.2
	18	3	20.8	4.3	11.6 - 29.5
	12	4	18.4	2.1	13.5 - 23.7
83 µg/g Cd	18	0	18.9	3.3	12.2 - 24.2
	18	1	17.4	4.1	11.1 - 25.9
	18	2	18.0	3.6	12.1 - 25.7
	18	3	17.0	3.9	11.8 - 24.7
	11	4	14.4	4.2	9.1 - 20.9

*N = Number of observations.

Table 16. Values of white blood cells (thousands/ μl) in young pigs (See Figure 19).

Group	N*	Day	Mean	Standard Deviation	Range
I	4	0	19.5	4.7	14.2 - 23.7
	4	2	18.3	6.7	10.0 - 26.5
	4	4	16.3	3.3	12.1 - 20.1
	4	6	21.1	5.5	16.0 - 26.0
	4	10	17.6	4.3	12.5 - 23.2
II	4	0	17.8	3.0	13.5 - 20.5
	4	2	14.8	3.3	10.2 - 18.0
	4	4	16.6	1.1	15.5 - 18.2
	4	6	17.8	1.7	16.5 - 20.2
	4	10	16.7	1.5	14.6 - 18.3
III	4	0	17.9	1.2	16.9 - 19.6
	4	2	15.3	4.0	9.6 - 18.7
	4	4	17.2	1.4	15.5 - 18.8
	4	6	18.1	0.7	17.4 - 19.1
	4	10	19.2	2.9	15.1 - 22.0
IV	3	0	11.0	2.0	9.1 - 13.2
	3	2	16.4	8.1	10.0 - 25.8
	3	4	14.0	3.7	11.3 - 18.3
	3	6	13.1	2.8	11.2 - 16.3
	3	10	12.6	1.5	11.6 - 14.4

Table 16 - continued

Group	N*	Day	Mean	Standard Deviation	Range
V	4	0	18.8	1.8	17.0 - 20.9
	4	2	14.9	1.3	13.5 - 16.8
	4	4	14.3	5.4	9.4 - 19.6
	4	6	17.1	7.0	10.5 - 27.0
	4	10	24.1	11.6	14.1 - 40.7
VI	4	0	12.6	3.5	9.5 - 17.5
	4	2	12.1	1.3	11.7 - 14.0
	4	4	12.3	2.2	9.5 - 15.1
	4	6	13.1	3.4	10.4 - 18.2
	2	10	14.1	0.3	13.9 - 14.3

*N = Number of observations.

Table 17. Values of alkaline phosphatase (IU/L) in young pigs (See Figure 15).

Group	N*	Week	Mean	Standard Deviation	Range
Control	18	0	226.55	53.66	147 - 349
	18	1	222.66	53.25	90 - 287
	18	2	259.94	44.09	159 - 314
	18	3	269.66	63.36	148 - 380
	12	4	271.25	62.17	144 - 363
83 µg/g Cd	18	0	269.11	72.10	161 - 472
	18	1	256.22	61.16	141 - 365
	18	2	266.22	63.62	149 - 401
	18	3	253.05	72.39	155 - 453
	11	4	225.18	56.38	90 - 309

*N = Number of observations.

Table 18. Values of alkaline phosphatase (IU/L) in young pigs (See Figure 16).

Group	N*	Day	Mean	Standard Deviation	Range
I	4	0	271.50	36.92	217 - 297
	4	2	254.25	50.16	180 - 289
	4	4	254.00	23.83	220 - 274
	4	6	233.25	24.11	205 - 263
	4	10	252.00	32.13	222 - 295
II	4	0	259.50	89.71	144 - 363
	4	2	431.50	213.34	195 - 648
	4	4	878.50	623.20	259 - 1,420
	4	6	803.50	578.61	171 - 1,490
	4	10	860.75	690.82	108 - 1,650
III	4	0	282.75	66.32	209 - 357
	4	2	264.00	50.19	211 - 323
	4	4	266.50	39.40	224 - 302
	4	6	245.75	38.75	226 - 292
	4	10	262.25	32.25	240 - 306
IV	3	0	216.66	27.61	193 - 247
	3	2	211.00	25.51	192 - 240
	3	4	218.66	32.62	183 - 247
	3	6	205.66	33.17	177 - 242
	3	10	213.66	42.19	180 - 261

Table 18 - continued

Group	N*	Day	Mean	Standard Deviation	Range
V	4	0	211.00	80.85	90 - 249
	4	2	265.00	128.74	83 - 381
	4	4	366.75	182.42	114 - 547
	4	6	360.75	167.94	121 - 498
	4	10	305.25	130.44	130 - 417
VI	4	0	245.75	51.45	183 - 309
	4	2	245.25	48.48	192 - 309
	4	4	247.75	56.54	206 - 330
	4	6	162.75	86.46	44 - 250
	2	10	257.00	59.39	215 - 299

*N = Number of observations.

Table 19. Values of sorbitol dehydrogenase (IU/L) in young pigs (See Figure 17).

Group	N*	Week	Mean	Standard Deviation	Range
Control	18	0	1.61	0.94	0.0 - 3.5
	18	1	1.90	1.92	0.0 - 6.8
	18	2	2.08	0.78	0.7 - 3.7
	18	3	3.11	1.67	0.8 - 5.9
	18	4	2.33	0.77	1.3 - 3.5
83 µg/g Cd	18	0	5.16	6.44	0.0 - 24.0
	18	1	1.93	2.89	0.2 - 12.8
	18	2	5.15	2.84	1.0 - 9.5
	18	3	3.92	2.12	0.8 - 6.8
	11	4	2.66	2.11	0.3 - 4.1

*N = Number of observations.

Table 20. Values of sorbitol dehydrogenase (IU/L) in young pigs (See Figure 18).

Group	N*	Day	Mean	Standard Deviation	Range
I	4	0	2.82	0.83	1.8 - 3.5
	4	2	1.35	0.36	1.0 - 1.8
	4	6	2.35	0.75	1.6 - 3.3
	4	6	1.32	0.46	0.7 - 1.8
	4	10	1.50	0.50	0.8 - 2.0
II	4	0	2.15	0.58	1.6 - 2.7
	4	2	31.25	33.74	10.4 - 81.2
	4	4	8.72	10.18	3.4 - 24.0
	4	6	6.35	7.97	1.9 - 18.3
	4	10	2.77	2.19	0.7 - 5.8
III	4	0	2.02	0.63	1.3 - 2.8
	4	2	8.87	0.35	8.6 - 9.4
	4	4	1.80	0.67	1.3 - 2.8
	4	6	0.95	0.34	0.5 - 1.3
	4	10	1.07	0.56	0.5 - 1.4
IV	3	0	2.26	1.68	0.8 - 4.1
	3	2	4.03	4.60	0.8 - 9.3
	3	4	2.80	0.36	2.5 - 3.2
	3	6	2.43	0.20	2.2 - 2.6
	3	10	1.53	0.06	1.5 - 1.6

Table 20 - continued

Group	N*	Day	Mean	Standard Deviation	Range
V	4	0	2.70	1.00	1.7 - 3.8
	4	2	21.35	38.17	1.5 - 78.6
	4	4	3.92	1.10	2.5 - 5.2
	4	6	3.17	2.09	1.3 - 5.7
	4	10	0.80	0.77	0.1 - 1.8
VI	4	0	2.92	3.42	0.3 - 7.7
	4	2	3.10	1.80	1.6 - 5.7
	4	4	2.32	1.66	0.6 - 4.6
	4	6	1.45	0.78	0.5 - 2.4
	2	10	1.10	0.14	1.0 - 1.2

*N = Number of observations.

Table 21. Values of aspartate aminotransferase (IU/L) in young pigs (See Figure 19).

Group	N*	Week	Mean	Standard Deviation	Range
Control	18	0	27.83	4.40	21.0 - 38.0
	18	1	22.05	5.03	14.0 - 33.0
	18	2	19.22	6.20	12.0 - 32.0
	18	3	19.16	3.38	14.0 - 23.0
	12	4	19.66	5.59	13.0 - 33.0
83 µg/g Cd	18	0	24.05	5.97	18.0 - 44.0
	18	1	21.55	5.85	9.0 - 32.0
	18	2	21.00	4.21	14.0 - 29.0
	18	3	20.88	5.35	15.0 - 37.0
	11	4	24.18	9.07	12.0 - 39.0

*N = Number of observations.

Table 22. Values of aspartate aminotransferase (IU/L) in young pigs (See Figure 20).

Group	N*	Day	Mean	Standard Deviation	Range
I	4	0	19.50	3.10	16.0 - 23.0
	4	2	17.75	3.86	14.0 - 23.0
	4	4	21.25	5.31	17.0 - 29.0
	4	6	18.25	2.50	17.0 - 22.0
	4	10	26.00	3.36	21.0 - 28.0
II	4	0	19.50	9.11	13.0 - 33.0
	4	2	144.25	202.11	20.0 - 446.0
	4	4	98.75	83.22	33.0 - 210.0
	4	6	57.00	41.25	28.0 - 118.0
	4	10	85.00	83.27	15.0 - 204.0
III	4	0	20.00	4.69	15.0 - 24.0
	4	2	62.00	51.96	18.0 - 133.0
	4	4	25.25	9.25	19.0 - 39.0
	4	6	21.25	6.50	18.0 - 31.0
	4	10	27.50	2.64	24.0 - 30.0
IV	3	0	15.00	2.64	12.0 - 17.0
	3	2	12.33	3.51	9.0 - 16.0
	3	4	15.33	2.08	13.0 - 17.0
	3	6	13.33	1.15	12.0 - 14.0
	3	10	28.00	13.89	19.0 - 44.0

Table 22 - continued

Group	N*	Day	Mean	Standard Deviation	Range
V	4	0	30.50	8.34	19.0 - 39.0
	4	2	69.25	87.85	23.0 - 201.0
	4	4	31.50	6.02	26.0 - 40.0
	4	6	28.00	3.16	24.0 - 31.0
	4	10	34.50	8.10	27.0 - 46.0
VI	4	0	24.75	7.88	18.0 - 36.0
	4	2	23.75	1.75	22.0 - 26.0
	4	4	24.00	4.08	19.0 - 29.0
	4	6	16.00	5.94	8.0 - 21.0
	2	10	24.50	3.53	22.0 - 27.0

*N = Number of observations.

Table 23. Values of blood urea nitrogen (mg/dl) in young pigs (See Figure 21).

Group	N*	Week	Mean	Standard Deviation	Range
Control	18	0	16.72	4.02	12 - 26
	18	1	14.11	3.59	14 - 33
	18	2	14.22	4.49	0 - 18
	18	3	15.22	2.57	12 - 20
	12	4	9.16	1.33	8 - 12
83 µg/g Cd	18	0	18.22	4.79	10 - 26
	18	1	12.77	2.75	9 - 32
	18	2	14.88	5.05	6 - 24
	18	3	13.00	2.40	8 - 16
	11	4	7.27	1.61	6 - 10

*N = Number of observations.

Table 24. Values of blood urea nitrogen (mg/dl) in young pigs (See Figure 22).

Group	N*	Day	Mean	Standard Deviation	Range
I	4	0	8.00	0.00	8 - 8
	4	2	15.00	2.00	14 - 18
	4	4	15.00	1.15	14 - 16
	4	6	14.50	1.91	12 - 16
	4	10	16.00	1.63	14 - 16
II	4	0	10.00	0.00	10 - 10
	4	2	11.00	2.00	8 - 12
	4	4	9.00	2.58	8 - 12
	4	6	11.00	4.76	6 - 16
	4	10	11.50	6.80	6 - 20
III	4	0	9.50	1.91	8 - 12
	4	2	16.50	3.00	14 - 20
	4	4	14.00	5.88	6 - 20
	4	6	13.50	1.91	12 - 16
	4	10	16.00	2.82	14 - 20
IV	3	0	6.66	1.15	6 - 8
	3	2	14.00	0.00	14 - 14
	3	4	13.33	2.30	12 - 16
	3	6	12.00	0.00	12 - 12
	3	10	13.33	1.15	12 - 14

Table 24 - continued

Group	N*	Day	Mean	Standard Deviation	Range
V	4	0	7.50	1.91	6 - 10
	4	2	11.50	3.41	8 - 16
	4	4	13.25	1.50	11 - 14
	4	6	10.00	5.41	8 - 18
	4	10	11.50	4.12	6 - 16
VI	4	0	7.50	1.91	6 - 10
	4	2	13.50	1.91	12 - 16
	4	4	16.00	6.32	10 - 24
	4	6	10.00	2.82	6 - 12
	2	10	13.00	1.41	12 - 14

*N = Number of observations.

Table 25. Values of prothrombin time (seconds) in young pigs (See Figure 23).

Group	N*	Week	Mean	Standard Deviation	Range
Control	18	0	15.87	1.53	13.3 - 19.8
	18	1	14.72	1.39	12.8 - 19.0
	18	2	14.17	1.71	11.3 - 18.3
	18	3	14.07	0.56	12.9 - 15.3
	12	4	13.83	0.60	13.3 - 15.4
83 ug/g Cd	18	0	15.36	1.09	13.3 - 16.8
	18	1	14.46	1.56	12.8 - 17.8
	18	2	14.71	3.73	12.3 - 28.3
	18	3	10.41	2.82	10.9 - 22.9
	11	4	12.58	0.63	11.8 - 13.4

*N = Number of observations.

Table 26. Values of prothrombin time (seconds) in young pigs (See Figure 24).

Group	N*	Day	Mean	Standard Deviation	Range
I	4	0	13.52	0.32	13.3 - 14.0
	4	2	13.65	0.50	12.9 - 13.9
	4	4	13.22	0.55	12.4 - 13.5
	4	6	13.57	0.61	12.9 - 14.4
	4	10	13.55	0.47	12.9 - 13.9
II	4	0	14.12	0.89	13.3 - 15.4
	4	2	15.52	1.25	13.9 - 16.9
	4	4	22.55	4.16	18.9 - 26.4
	4	6	30.37	10.06	16.9 - 39.9
	4	10	63.65	36.92	16.5 - 103.8
III	4	0	13.85	0.45	13.3 - 14.4
	4	2	21.97	7.23	13.9 - 31.4
	4	4	27.62	4.91	20.8 - 31.9
	4	6	39.15	12.65	27.9 - 52.9
	4	10	15.42	1.05	14.5 - 16.9
IV	3	0	12.40	0.10	12.3 - 12.5
	3	2	12.46	0.55	11.9 - 13.0
	3	4	12.36	0.11	12.3 - 12.5
	3	6	12.93	0.45	12.5 - 13.4
	3	10	12.56	0.70	11.9 - 13.3

Table 26 - continued

Group	N*	Day	Mean	Standard Deviation	Range
V	4	0	12.72	0.74	11.9 - 13.4
	4	2	13.52	2.17	11.3 - 16.9
	4	4	14.85	3.60	11.3 - 19.8
	4	6	16.12	3.78	12.5 - 21.3
	4	10	14.20	2.54	11.0 - 16.9
VI	4	0	12.57	0.84	11.8 - 13.4
	4	2	26.05	4.25	23.4 - 32.4
	4	4	44.52	17.42	32.4 - 69.9
	4	6	67.92	45.75	33.9 - 135.4
	2	10	13.95	0.63	13.5 - 14.4

*N = Number of observations.

Table 27. Values of activated partial thromboplastin time (seconds) in young pigs (See Figure 25).

Group	N*	Week	Mean	Standard Deviation	Range
Control	18	0	35.30	5.13	29.4 - 47.5
	18	1	34.28	3.87	29.3 - 45.4
	18	2	33.47	5.17	27.3 - 48.4
	18	3	30.88	4.13	25.9 - 39.4
	12	4	28.49	1.63	26.5 - 31.3
83 µg/g Cd	18	0	35.32	3.61	29.5 - 43.3
	18	1	35.72	3.21	29.4 - 43.7
	18	2	36.81	8.72	26.3 - 49.4
	18	3	33.17	6.89	25.4 - 49.5
	11	4	27.80	1.69	26.9 - 31.5

*N = Number of observations.

Table 28. Values of activated partial thromboplastin time (seconds) in young pigs (See Figure 26).

Group	N*	Day	Mean	Standard Deviation	Range
I	4	0	29.50	1.20	28.8 - 31.3
	4	2	26.20	3.16	24.0 - 30.9
	4	4	27.67	3.80	22.8 - 30.9
	4	6	26.15	2.08	23.9 - 28.9
	4	10	29.55	3.84	24.9 - 32.5
II	4	0	28.92	1.63	26.5 - 29.9
	4	2	31.25	3.77	27.9 - 36.3
	4	4	30.40	2.34	27.9 - 33.4
	4	6	39.40	13.02	28.9 - 57.9
	4	10	48.40	20.27	28.9 - 65.9
III	4	0	27.05	1.13	25.5 - 27.9
	4	2	37.50	7.46	26.4 - 42.3
	4	4	55.10	7.80	44.9 - 63.9
	4	6	82.65	29.34	63.9 - 125.9
	4	10	32.15	3.59	26.9 - 34.9
IV	3	0	27.60	0.36	27.3 - 28.0
	3	2	26.10	1.05	24.9 - 26.9
	3	4	27.26	1.09	26.4 - 28.5
	3	6	30.23	4.04	25.9 - 33.9
	3	10	32.40	0.50	31.9 - 32.9

Table 28 - continued

Group	N*	Day	Mean	Standard Deviation	Range
V	4	0	28.17	2.88	26.9 - 31.5
	4	2	24.67	3.77	21.4 - 29.9
	4	4	28.02	2.46	24.4 - 29.9
	4	6	24.40	3.71	21.4 - 29.5
	4	10	18.30	2.02	16.5 - 20.9
VI	4	0	27.60	0.94	26.9 - 29.0
	4	2	40.77	14.39	24.3 - 55.4
	4	4	57.57	15.22	39.9 - 71.5
	4	6	120.65	107.73	47.9 - 280.9
	2	10	30.20	4.66	26.9 - 33.5

*N = Number of observations.

Table 29. Values of fibrinogen (mg/100 ml) in young pigs (See Figure 27).

Group	N*	Week	Mean	Standard Deviation	Range
Control	18	0	283.33	78.59	200 - 400
	18	1	316.66	120.04	100 - 500
	18	2	272.22	112.74	100 - 400
	18	3	233.33	113.75	100 - 500
	12	4	308.33	137.89	100 - 500
83 µg/g Cd	18	0	283.33	78.59	200 - 400
	18	1	238.88	97.85	100 - 400
	18	2	205.55	87.26	100 - 400
	18	3	344.94	150.38	100 - 600
	11	4	218.18	107.87	100 - 400

*N = Number of observations.

Table 30. Values of fibrinogen (mg/100 ml) in young pigs (See Figure 28).

Group	N*	Day	Mean	Standard Deviation	Range
I	4	0	250.00	129.09	100 - 400
	4	2	225.00	50.00	200 - 300
	4	4	250.00	57.73	200 - 300
	4	6	200.00	81.64	100 - 300
	4	10	350.00	173.20	200 - 600
II	4	0	275.00	170.78	100 - 500
	4	2	250.00	57.73	200 - 300
	4	4	225.00	95.74	100 - 300
	4	6	225.00	150.00	100 - 400
	4	10	75.00	95.74	000 - 200
III	4	0	400.00	81.64	300 - 500
	4	2	175.00	50.00	100 - 200
	4	4	250.00	57.73	200 - 300
	4	6	350.00	57.73	300 - 400
	4	10	250.00	57.73	200 - 300
IV	3	0	233.33	115.47	100 - 300
	3	2	233.33	57.73	200 - 300
	3	4	400.00	100.00	300 - 500
	3	6	166.66	57.73	100 - 200
	3	10	400.00	200.00	200 - 600

Table 30 - continued

Group	N*	Day	Mean	Standard Deviation	Range
V	4	0	150.00	57.73	100 - 200
	4	2	200.00	0.00	200 - 200
	4	4	350.00	129.09	200 - 500
	4	6	200.00	81.64	100 - 300
	4	10	275.00	95.74	200 - 400
VI	4	0	275.00	125.83	100 - 400
	4	2	275.00	95.74	200 - 400
	4	4	225.00	150.00	100 - 400
	4	6	275.00	150.00	200 - 500
	2	10	300.00	141.42	200 - 400

*N = Number of observations.

Table 31. Values of serum total protein (g/dl) in young pigs (See Figure 29).

Group	N*	Week	Mean	Standard Deviation	Range
Control	18	0	5.96	0.88	4.80 - 7.60
	18	1	5.47	0.51	4.30 - 6.50
	18	2	5.83	0.43	5.20 - 6.60
	18	3	5.86	0.41	5.10 - 6.50
	12	4	5.91	0.47	4.88 - 6.67
83 µg/g Cd	18	0	5.99	0.63	5.10 - 7.00
	18	1	5.53	0.39	4.80 - 6.20
	18	2	5.66	0.38	5.10 - 6.30
	18	3	5.86	0.32	5.20 - 6.40
	11	4	6.00	0.30	5.37 - 6.40

*N = Number of observations.

Table 32. Values of serum total protein (g/dl) in young pigs (See Figure 30).

Group	N*	Day	Mean	Standard Deviation	Range
I	4	0	5.87	0.21	5.67 - 6.17
	4	2	6.06	0.29	5.75 - 6.38
	4	4	5.98	0.11	5.84 - 6.13
	4	6	6.00	0.06	5.97 - 6.10
	4	10	6.08	0.25	5.78 - 6.32
II	4	0	5.84	0.74	4.88 - 6.57
	4	2	6.07	0.61	5.26 - 6.68
	4	4	6.21	0.52	5.58 - 6.86
	4	6	6.09	0.82	4.97 - 6.84
	4	10	5.36	1.02	4.48 - 6.61
III	4	0	6.02	0.45	5.57 - 6.67
	4	2	5.90	0.59	5.42 - 6.67
	4	4	6.12	0.30	5.93 - 6.57
	4	6	6.02	0.36	5.80 - 6.47
	4	10	6.58	0.25	6.31 - 6.61
IV	3	0	5.94	0.52	5.37 - 6.40
	3	2	6.01	0.48	5.47 - 6.40
	3	4	5.83	0.42	5.35 - 6.08
	3	6	6.03	0.46	5.57 - 6.48
	3	10	6.10	0.42	5.77 - 6.58

Table 32 - continued

Group	N*	Day	Mean	Standard Deviation	Range
V	4	0	6.00	0.23	5.68 - 6.25
	4	2	6.14	0.34	5.74 - 6.58
	4	4	6.19	0.15	6.06 - 6.36
	4	6	6.62	0.12	6.47 - 6.77
	4	10	6.67	0.25	6.38 - 6.98
VI	4	0	6.04	0.24	5.68 - 6.19
	4	2	6.30	0.15	6.12 - 6.48
	4	4	6.43	0.18	6.18 - 6.57
	4	6	6.40	0.17	6.25 - 6.54
	2	10	6.46	0.01	6.45 - 6.47

*N = Number of observations.

Table 33. Values of serum albumin (g/dl) in young pigs (See Figure 31).

Group	N*	Day	Mean	Standard Deviation	Range
I	4	0	3.19	0.10	3.07 - 3.32
	4	2	3.22	0.46	2.62 - 3.73
	4	4	3.20	0.07	3.13 - 3.28
	4	6	3.27	0.37	2.72 - 3.49
	4	10	3.15	0.10	3.01 - 3.25
II	4	0	3.04	0.61	2.28 - 3.71
	4	2	3.01	0.49	2.32 - 3.45
	4	4	3.10	0.43	2.71 - 3.56
	4	6	3.37	0.47	2.77 - 3.93
	4	10	3.68	1.11	3.35 - 5.27
III	4	0	3.02	0.39	2.79 - 3.55
	4	2	3.30	0.47	2.87 - 3.88
	4	4	3.18	0.39	2.76 - 3.67
	4	6	2.93	0.27	2.55 - 3.22
	4	10	3.43	0.33	3.05 - 3.80
IV	3	0	3.02	0.54	2.50 - 3.59
	3	2	3.07	0.17	2.87 - 3.18
	3	4	2.72	0.30	2.40 - 3.00
	3	6	3.01	0.25	2.76 - 3.27
	3	10	2.84	0.25	2.61 - 3.12

Table 33 - continued

Group	N*	Day	Mean	Standard Deviation	Range
V	4	0	2.66	0.13	2.46 - 2.75
	4	2	3.17	0.60	2.32 - 3.66
	4	4	2.87	0.31	2.48 - 3.15
	4	6	3.20	0.30	2.80 - 3.45
	4	10	2.97	0.26	2.73 - 3.33
VI	4	0	3.04	0.19	2.77 - 3.22
	4	2	3.28	0.37	2.90 - 3.78
	4	4	2.94	0.13	2.74 - 3.02
	4	6	2.90	0.16	2.73 - 3.07
	2	10	2.97	0.18	2.84 - 3.10

*N = Number of observations.

Table 34. Values of serum alpha globulin (g/dl) in young pigs (See Figure 32).

Group	N*	Day	Mean	Standard Deviation	Range
I	4	0	1.03	0.08	0.93 - 1.13
	4	2	1.09	0.17	0.92 - 1.30
	4	4	1.03	0.07	0.95 - 1.12
	4	6	0.93	0.15	0.79 - 1.09
	4	10	1.03	0.03	0.98 - 1.05
II	4	0	1.01	0.06	0.92 - 1.07
	4	2	1.15	0.10	1.02 - 1.26
	4	4	1.18	0.24	0.87 - 1.45
	4	6	0.91	0.20	0.72 - 1.18
	4	10	0.53	0.56	0.00 - 1.17
III	4	0	1.02	0.10	0.87 - 1.13
	4	2	0.72	0.39	0.80 - 1.06
	4	4	0.99	0.28	0.74 - 1.40
	4	6	0.98	0.05	0.95 - 1.07
	4	10	0.97	0.09	0.84 - 1.05
IV	3	0	1.13	0.08	1.06 - 1.23
	3	2	1.06	0.06	1.01 - 1.13
	3	4	1.16	0.08	1.07 - 1.23
	3	6	1.15	0.05	1.08 - 1.17
	3	10	1.17	0.09	1.09 - 1.28

Table 34 - continued

Group	N*	Day	Mean	Standard Deviation	Range
V	4	0	1.28	0.16	1.13 - 1.48
	4	2	1.19	0.19	0.01 - 1.44
	4	4	1.28	0.21	1.14 - 1.59
	4	6	1.30	0.26	1.02 - 1.66
	4	10	1.22	0.21	1.01 - 1.52
VI	4	0	1.10	0.09	0.97 - 1.19
	4	2	1.09	0.18	0.97 - 1.34
	4	4	1.21	0.05	1.16 - 1.29
	4	6	1.30	0.19	1.04 - 1.48
	2	10	1.16	0.14	1.06 - 1.26

*N = Number of observations.

Table 35. Values of serum beta globulin (g/dl) in young pigs (See Figure 33).

Group	N*	Day	Mean	Standard Deviation	Range
I	4	0	0.98	0.09	0.85 - 1.07
	4	2	1.00	0.04	0.93 - 1.04
	4	4	1.10	0.14	0.98 - 1.12
	4	6	1.09	0.08	1.00 - 1.21
	4	10	1.15	0.16	0.96 - 1.32
II	4	0	1.13	0.08	1.01 - 1.20
	4	2	1.08	0.06	1.00 - 1.16
	4	4	0.95	0.17	0.80 - 1.19
	4	6	0.82	0.23	0.64 - 1.16
	4	10	0.63	0.23	0.52 - 0.84
III	4	0	1.12	0.13	0.94 - 1.23
	4	2	1.13	0.21	0.91 - 1.43
	4	4	0.99	0.14	0.83 - 1.17
	4	6	1.20	0.09	1.10 - 1.33
	4	10	1.21	0.19	0.94 - 1.41
IV	3	0	1.18	0.18	1.00 - 1.36
	3	2	1.30	0.21	1.10 - 1.52
	3	4	1.21	0.14	1.10 - 1.38
	3	6	1.48	0.33	1.15 - 1.82
	3	10	1.27	0.18	1.10 - 1.46

Table 35 - continued

Group	N*	Day	Mean	Standard Deviation	Range
V	4	0	1.11	0.19	0.84 - 1.31
	4	2	1.08	0.15	0.87 - 1.23
	4	4	1.03	0.17	0.90 - 1.22
	4	6	1.13	0.18	0.93 - 1.32
	4	10	1.16	0.14	0.97 - 1.30
VI	4	0	1.17	0.05	1.12 - 1.23
	4	2	1.24	0.09	1.13 - 1.37
	4	4	1.35	0.07	1.28 - 1.46
	4	6	1.34	0.08	1.27 - 1.47
	2	10	1.32	0.08	1.26 - 1.38

*N = Number of observations.

Table 36. Values of serum gamma globulin (g/dl) in young pigs (See Figure 34)

Group	N*	Day	Mean	Standard Deviation	Range
I	4	0	0;68	0.23	0.46 - 0.99
	4	2	0.73	0.05	0.67 - 0.79
	4	4	0.65	0.23	0.39 - 0.96
	4	6	0.70	0.31	0.50 - 1.16
	4	10	0.75	0.18	0.59 - 0.96
II	4	0	0.63	0.18	0.42 - 0.82
	4	2	0.82	0.18	0.61 - 1.02
	4	4	0.97	0.27	0.59 - 1.20
	4	6	0.97	0.37	0.42 - 1.21
	4	10	0.51	0.53	0.00 - 1.15
III	4	0	0.85	0.08	0.76 - 0.95
	4	2	0.74	0.13	0.56 - 0.87
	4	4	0.94	0.08	0.82 - 0.99
	4	6	0.90	0.18	0.73 - 1.12
	4	10	0.95	0.13	0.80 - 1.10
IV	3	0	0.60	0.32	0.39 - 0.98
	3	2	0.57	0.31	0.26 - 0.89
	3	4	0.73	0.24	0.56 - 1.02
	3	6	0.42	0.26	0.15 - 0.68
	3	10	0.81	0.18	0.62 - 0.99

Table 36 - continued

Group	N*	Day	Mean	Standard Deviation	Range
V	4	0	0.95	0.11	0.78 - 1.02
	4	2	0.70	0.17	0.45 - 0.81
	4	4	1.00	0.20	0.81 - 1.16
	4	6	0.98	0.20	0.80 - 1.27
	4	10	1.29	0.24	0.95 - 1.49
VI	4	0	0.75	0.10	0.62 - 0.86
	4	2	0.68	0.27	0.28 - 0.88
	4	4	0.91	0.05	0.87 - 0.98
	4	6	0.85	0.05	0.78 - 0.92
	2	10	1.01	0.02	0.99 - 1.03

*N = Number of observations.

Table 37. Values of cadmium concentration (µg/g-wet digestion) in tissue and urine (See Figures 35 and 36).

Group	N*	Slaughter	Sample	Mean	Standard Deviation	Range
Control	6	1 (5/4/79)	kidney	0.00	-	-
			liver	0.00	-	-
			muscle	0.00	-	-
			urine	0.00	-	-
83 µg/g Cd diet	6	1	kidney	35.13	18.21	17.70 - 63.80
			liver	6.80	1.92	3.48 - 10.90
			muscle	< 0.22	0.10	0.12 - 0.44
			urine	0.02	0.006	0.01 - 0.06
Control	12	2 (5/18/79)	kidney	0.00	-	-
			liver	0.00	-	-
			muscle	0.00	-	-
			urine	0.00	-	-
83 µg/g Cd diet	12	2	kidney	42.90	10.57	22.20 - 59.20
			liver	7.90	2.37	3.20 - 13.70
			muscle	0.09	0.07	0.00 - 0.35
			urine	0.02	0.02	0.00 - 0.09

*N = Number of observations.

Table 38. Values of cadmium concentration (µg/g-wet digestion) in tissue (See Figure 37).

Group	N*	Slaughter	Sample	Mean	Standard Deviation	Range
I	2	1 (5/4/79)	kidney	0.00	-	-
			liver	0.00	-	-
			muscle	0.00	-	-
II	2	1	kidney	0.00	-	-
			liver	0.00	-	-
			muscle	0.00	-	-
III	2	1	kidney	0.00	-	-
			liver	0.00	-	-
			muscle	0.00	-	-
IV	2	1	kidney	28.05	5.58	24.10 - 32.00
			liver	4.54	1.49	3.48 - 5.60
			muscle	0.15	0.01	0.14 - 0.16
V	2	1	kidney	32.85	21.42	17.70 - 48.00
			liver	7.50	0.70	7.00 - 8.00
			muscle	0.22	0.09	0.15 - 0.29
VI	2	1	kidney	44.50	27.29	25.20 - 63.80
			liver	8.37	3.57	5.85 - 10.90
			muscle	0.28	0.22	0.12 - 0.44

Table 38 - continued

Group	N*	Slaughter	Sample	Mean	Standard Deviation	Range
I	4	2 (5/18/79)	kidney	0.00	-	-
			liver	0.00	-	-
			muscle	0.00	-	-
II	4	2	kidney	0.00	-	-
			liver	0.00	-	-
			muscle	0.00	-	-
III	4	2	kidney	0.00	-	-
			liver	0.00	-	-
			muscle	0.00	-	-
IV	4	2	kidney	37.47	13.70	22.20 - 55.50
			liver	8.22	4.29	3.20 - 13.70
			muscle	0.06	0.03	0.04 - 0.11
V	4	2	kidney	48.25	9.99	38.00 - 59.20
			liver	7.55	1.04	6.30 - 8.60
			muscle	0.11	0.15	0.00 - 0.34
VI	4	2	kidney	43.00	8.02	31.60 - 50.40
			liver	8.00	1.80	6.40 - 9.80
			muscle	0.10	0.04	0.04 - 0.14

*N = Number of observations.

Table 39. Values of iron concentration (µg/g-wet digestion) in tissue and urine (See Figures 38 and 39).

Group	N*	Slaughter	Sample	Mean	Standard Deviation	Range
Control	6	1 (5/4/79)	kidney	34.90	16.91	1.17 - 5.92
			liver	128.96	48.57	57.8 - 245.0
			muscle	9.93	2.04	9.9 - 14.0
			urine	0.05	0.00	0.10 - 0.10
83 µg/g Cd	6	1	kidney	31.66	8.71	2.40 - 5.27
			liver	63.75	52.80	24.0 - 235.0
			muscle	9.93	2.21	7.2 - 14.8
			urine	0.05	0.02	0.00 - 0.10
Control	12	2 (5/13/79)	kidney	41.55	12.61	2.55 - 5.78
			liver	144.55	77.96	27.3 - 344.0
			muscle	14.58	3.26	9.9 - 20.9
			urine	0.10	0.05	0.00 - 0.30
83 µg/g Cd	12	2	kidney	35.26	12.06	2.56 - 7.40
			liver	39.73	19.62	21.7 - 115.0
			muscle	13.99	4.22	8.6 - 28.0
			urine	0.03	0.05	0.00 - 0.10

*N = Number of observations.

Table 40. Values of iron concentration (µg/g-wet digestion) in tissue (See Figure 40).

Group	N*	Slaughter	Sample	Mean	Standard Deviation	Range
I	2	1 (5/4/79)	kidney	30.55	26.65	1.17 - 4.94
			liver	159.00	11.31	151.0 - 164.0
			muscle	10.80	0.84	10.2 - 11.4
II	2	1	kidney	46.60	17.81	3.40 - 5.92
			liver	159.90	120.34	74.8 - 245.0
			muscle	11.95	2.89	9.9 - 14.0
III	2	1	kidney	27.55	6.29	2.31 - 3.20
			liver	68.00	14.42	57.8 - 78.2
			muscle	11.90	2.40	10.2 - 13.6
IV	2	1	kidney	27.60	1.69	2.64 - 2.88
			liver	28.90	6.22	24.5 - 33.3
			muscle	11.10	0.84	10.5 - 11.7
V	2	1	kidney	38.35	20.29	2.40 - 5.27
			liver	129.50	149.19	24.0 - 235.0
			muscle	11.00	5.37	7.2 - 14.8
VI	2	1	kidney	29.05	4.17	2.61 - 3.20
			liver	32.85	3.18	30.6 - 35.1
			muscle	7.75	0.42	7.4 - 8.0

Table 40 - continued

Group	N*	Slaughter	Sample	Mean	Standard Deviation	Range
I	4	2 (5/18/79)	kidney	48.40	11.24	3.50 - 5.78
			liver	127.75	47.98	75.6 - 170.0
			muscle	14.42	0.62	13.8 - 15.1
II	4	2	kidney	37.72	12.78	2.63 - 5.32
			liver	117.90	151.48	27.3 - 344.0
			muscle	13.75	4.99	9.9 - 20.9
III	4	2	kidney	38.55	13.81	2.55 - 5.70
			liver	128.00	34.91	76.0 - 151.0
			muscle	15.75	4.18	11.1 - 19.8
IV	4	2	kidney	39.82	23.15	2.45 - 7.40
			liver	52.40	41.81	27.8 - 115.0
			muscle	17.40	9.14	8.6 - 28.0
V	4	2	kidney	30.30	4.06	2.70 - 3.60
			liver	30.47	8.05	21.7 - 40.7
			muscle	11.92	1.22	10.8 - 13.3
VI	4	2	kidney	35.67	8.98	2.56 - 4.68
			liver	36.32	9.02	29.1 - 49.4
			muscle	12.62	2.31	10.2 - 14.8

*N = Number of observations.

Table 41. Values of zinc concentration (ug/g-wet digestion) in tissue and urine (See Figures 41 and 42).

Group	N*	Slaughter	Sample	Mean	Standard Deviation	Range
Control	6	1 (5/4/79)	kidney	20.91	2.19	18.4 - 23.3
			liver	38.96	14.93	24.4 - 71.4
			muscle	13.58	1.67	11.7 - 18.0
			urine	0.72	0.18	0.3 - 1.30
83 ug/g Cd	6	1	kidney	36.35	8.44	26.0 - 48.0
			liver	32.73	9.28	16.6 - 44.2
			muscle	12.33	2.73	10.1 - 17.0
			urine	0.86	0.32	0.5 - 1.7
Control	12	2 (5/18/79)	kidney	21.35	1.89	14.8 - 24.9
			liver	38.78	10.88	18.4 - 54.0
			muscle	15.04	3.39	10.6 - 22.4
			urine	0.92	0.38	0.2 - 2.10
83 ug/g Cd	12	2	kidney	37.25	3.53	31.10 - 44.00
			liver	35.31	6.98	20.8 - 49.4
			muscle	17.61	7.10	9.8 - 51.0
			urine	0.82	0.24	0.5 - 13.

*N = Number of observations.

Table 42. Values of zinc concentration (ug/g-wet digestion) in tissue (See Figure 43).

Group	N*	Slaughter	Sample	Mean	Standard Deviation	Range
I	2	1 (5/4/79)	kidney	20.95	0.07	20.9 - 21.0
			liver	36.40	9.61	29.6 - 43.2
			muscle	11.95	0.35	11.7 - 12.2
II	2	1	kidney	20.95	3.04	18.8 - 23.10
			liver	29.20	6.78	24.4 - 34.0
			muscle	13.70	0.56	13.3 - 14.1
III	2	1	kidney	20.85	3.46	18.4 - 23.3
			liver	51.30	28.42	31.2 - 71.4
			muscle	15.10	4.10	12.2 - 18.0
IV	2	1	kidney	35.35	4.31	32.3 - 38.4
			liver	23.15	9.26	16.6 - 29.7
			muscle	12.40	3.25	10.1 - 14.7
V	2	1	kidney	37.02	15.52	26.0 - 48.0
			liver	36.00	11.31	28.0 - 44.0
			muscle	14.05	4.17	11.1 - 17.0
VI	2	1	kidney	36.70	5.51	32.8 - 40.6
			liver	39.05	7.28	33.9 - 44.2
			muscle	10.55	0.77	10.0 - 11.1

Table 42 - continued

Group	N*	Slaughter	Sample	Mean	Standard Deviation	Range
I	4	2 (5/13/79)	kidney	23.27	1.18	22.4 - 24.9
			liver	44.50	9.66	33.3 - 53.3
			muscle	15.95	4.46	10.6 - 21.5
II	4	2	kidney	19.67	3.84	14.8 - 24.0
			liver	28.52	14.19	18.4 - 49.4
			muscle	16.10	4.71	11.2 - 22.4
III	4	2	kidney	21.10	0.67	20.6 - 22.1
			liver	43.32	8.79	34.5 - 54.0
			muscle	13.07	1.00	11.9 - 14.1
IV	4	2	kidney	35.62	3.95	31.1 - 40.7
			liver	39.35	7.14	32.6 - 49.4
			muscle	11.37	1.47	9.8 - 13.3
V	4	2	kidney	38.40	3.99	34.6 - 44.0
			liver	33.20	4.74	27.3 - 37.7
			muscle	13.82	1.61	11.5 - 15.2
VI	4	2	kidney	37.75	3.22	33.4 - 40.7
			liver	33.40	9.08	20.8 - 41.8
			muscle	27.65	18.23	11.9 - 51.0

*N = Number of observations.

Table 43. Values of copper concentration (µg/g-wet digestion) in tissue and urine (See Figures 44 and 45).

Group	N*	Slaughter	Sample	Mean	Standard Deviation	Range
Control	6	1 (5/4/79)	kidney	5.45	1.85	3.4 - 7.3
			liver	3.63	0.70	2.5 - 4.4
			muscle	0.86	0.14	0.7 - 1.1
			urine	0.05	0.00	0.05 - 0.06
83 µg/g Cd	6	1	kidney	9.03	4.80	4.6 - 17.6
			liver	4.08	1.29	1.5 - 7.4
			muscle	0.75	0.21	0.5 - 1.1
			urine	0.10	0.06	0.04 - 0.28
Control	12	2 (5/18/79)	kidney	6.37	1.48	4.0 - 11.4
			liver	4.36	0.80	3.1 - 6.5
			muscle	0.76	0.16	0.4 - 1.0
			urine	0.05	0.03	0.01 - 0.12
83 µg/g Cd	12	2	kidney	10.51	3.19	6.6 - 19.1
			liver	3.26	0.62	2.3 - 4.2
			muscle	0.68	0.19	0.4 - 0.8
			urine	0.09	0.05	0.05 - 0.13

*N = Number of observations.

Table 44. Values of copper concentration (µg/g-wet digestion) in tissue (See Figure 46).

Group	N*	Slaughter	Sample	Mean	Standard Deviation	Range
I	2	1 (5/4/79)	kidney	5.35	1.76	4.1 - 6.6
			liver	4.05	0.35	3.8 - 4.3
			muscle	0.80	0.00	0.8 - 0.8
II	2	1	kidney	4.90	2.12	3.4 - 6.4
			liver	3.40	0.42	3.1 - 3.7
			muscle	0.85	0.21	0.7 - 1.0
III	2	1	kidney	6.10	1.69	4.9 - 7.3
			liver	3.45	1.34	2.5 - 4.4
			muscle	0.95	0.21	0.8 - 1.1
IV	2	1	kidney	6.60	0.98	5.9 - 7.3
			liver	2.15	0.91	1.5 - 2.8
			muscle	0.85	0.35	0.6 - 1.1
V	2	1	kidney	8.85	5.51	4.6 - 12.4
			liver	4.50	0.42	4.2 - 4.8
			muscle	0.75	0.07	0.7 - 0.8
VI	2	1	kidney	12.00	7.91	6.4 - 17.6
			liver	5.60	2.54	3.8 - 7.4
			muscle	0.65	0.21	0.5 - 0.8

Table 44 - continued

Group	N*	Slaughter	Sample	Mean	Standard Deviation	Range
I	4	2 (5/18/79)	kidney	5.84	1.14	4.7 - 6.8
			liver	4.29	0.57	3.5 - 4.9
			muscle	0.78	0.15	0.6 - 1.0
II	4	2	kidney	7.73	3.13	4.0 - 11.4
			liver	5.11	1.31	3.5 - 6.5
			muscle	0.81	0.18	0.7 - 1.0
III	4	2	kidney	5.55	0.19	5.4 - 5.8
			liver	3.70	0.54	3.1 - 4.3
			muscle	0.70	0.20	0.4 - 0.8
IV	4	2	kidney	10.00	3.88	6.6 - 15.6
			liver	3.50	0.62	2.7 - 4.2
			muscle	0.65	0.19	0.4 - 0.8
V	4	2	kidney	12.10	4.88	8.6 - 19.1
			liver	3.00	0.66	2.3 - 3.9
			muscle	0.70	0.20	0.4 - 0.8
VI	4	2	kidney	9.45	0.83	8.3 - 10.3
			liver	3.30	0.58	2.7 - 4.1
			muscle	0.70	0.20	0.4 - 0.8

*N = Number of observations.

Table 45. Values of calcium concentration (µg/g-wet digestion) in tissue and urine (See Figures 47 and 48).

Group	N*	Slaughter	Sample	Mean	Standard Deviation	Range
Control	6	1 (5/4/79)	kidney	14.56	2.21	10.2 - 17.1
			liver	9.85	3.93	5.1 - 14.6
			muscle	10.23	2.45	8.2 - 17.3
			urine	33.33	30.15	17.0 - 84.0
83 ug/g Cd	6	1	kidney	15.20	3.94	11.8 - 23.0
			liver	7.85	2.27	2.9 - 11.2
			muscle	9.61	0.51	7.0 - 13.7
			urine	16.83	11.07	4.0 - 43.0
Control	12	2 (5/18/79)	kidney	11.61	2.40	8.8 - 15.9
			liver	9.34	5.37	2.3 - 28.9
			muscle	4.62	1.59	1.8 - 6.7
			urine	12.13	6.98	1.9 - 20.0
83 ug/g Cd	12	2	kidney	12.61	1.61	10.0 - 15.6
			liver	9.06	3.55	3.4 - 19.0
			muscle	5.70	1.86	1.8 - 9.8
			urine	11.16	7.66	1.4 - 28.0

*N = Number of observations

Table 46. Values of calcium concentration (µg/g-wet digestion) in tissue (See Figure 49).

Group	N*	Slaughter	Sample	Mean	Standard Deviation	Range
I	2	1 (5/4/79)	kidney	16.45	0.91	15.8 - 17.1
			liver	8.30	0.98	7.6 - 9.0
			muscle	9.13	0.55	8.7 - 9.5
II	2	1	kidney	14.45	2.05	13.0 - 15.9
			liver	11.40	4.10	7.0 - 8.5
			muscle	8.84	0.36	8.5 - 9.1
III	2	1	kidney	12.80	3.67	10.2 - 15.4
			liver	9.85	6.71	5.1 - 14.6
			muscle	12.73	6.46	8.2 - 17.3
IV	2	1	kidney	13.80	2.82	11.8 - 15.8
			liver	4.98	2.85	2.9 - 7.0
			muscle	13.50	0.28	13.3 - 13.7
V	2	1	kidney	17.70	7.49	12.4 - 23.0
			liver	9.40	2.54	7.6 - 11.2
			muscle	7.83	1.13	7.0 - 8.6
VI	2	1	kidney	14.12	1.52	13.0 - 15.2
			liver	9.19	1.42	8.2 - 10.2
			muscle	7.50	0.14	7.4 - 7.6

Table 46 - continued

Group	N*	Slaughter	Sample	Mean	Standard Deviation	Range
I	4	2 (5/18/79)	kidney	12.70	2.58	10.1 - 15.9
			liver	10.67	2.76	7.0 - 13.0
			muscle	5.47	1.17	4.2 - 6.7
II	4	2	kidney	11.02	3.02	8.8 - 15.5
			liver	12.35	11.42	2.3 - 28.9
			muscle	4.27	1.15	2.6 - 5.1
III	4	2	kidney	11.12	1.60	9.9 - 13.4
			liver	5.00	1.94	2.6 - 7.3
			muscle	4.12	2.45	1.8 - 6.3
IV	4	2	kidney	11.50	1.35	10.0 - 13.0
			liver	9.77	1.15	8.2 - 10.9
			muscle	5.57	2.62	1.8 - 7.7
V	4	2	kidney	12.05	2.20	10.3 - 15.2
			liver	6.60	2.05	4.9 - 9.3
			muscle	5.32	0.42	4.7 - 5.6
VI	4	2	kidney	14.30	1.29	13.0 - 15.6
			liver	7.82	7.47	3.4 - 19.0
			muscle	6.22	2.55	4.1 - 9.8

*N = Number of observations.

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BIOGRAPHICAL SKETCH

Orlando Osuna was born April 22, 1945, in Bogotá, Colombia, South America. In November, 1963, he finished high school at the Colegio Mayor de San Bartolomé (Sacerdotes Jesuitas) in Bogotá. He received the degree of Doctor of Veterinary Medicine and Animal Science from the Universidad Nacional de Colombia in December, 1969. The doctoral veterinary thesis was entitled, "Microbiologia de la Flora Vaginal Normal en Vacas Horras."

He has been enrolled as Assistant Professor of the College of Veterinary Medicine, Universidad Nacional de Colombia, since January, 1970. He taught Animal and Bovine Anatomy until June, 1971, and, from that time until September, 1972, was selected as Counterpart of the FAO-toxicologist expert, with whom he carried out research work in "falling sickness of the bovine" and "seleniosis." He participated in the Animal Toxicology courses for students in Veterinary and Pharmacy Sciences and developed the Toxicology Curriculum Planning presented to the First Teaching Veterinary Symposium of Colombia. During these two years, he took courses in "Teaching Methodology" given by ICCA-CIRA and "Food Hygiene" given by Panamerican Health Organization experts.

In October, 1972, he went to Washington, D.C., to attend the American Language Institute, Georgetown University. He

entered the graduate program at the University of Florida sponsored by FAO and under the direction of Dr. George T. Edds in January, 1973. He received the Master of Science in Veterinary Science (Toxicology) in December, 1974. His thesis was recommended for the Best Master's Thesis Award of the Year and was entitled, "The Toxic Effects of Aflatoxin B_1 in Male Holstein Calves With or Without Prior Infection by Flukes (Fasciola hepatica)." Early in this year, he participated in a 50 hour workshop in broad areas of veterinary toxicology at College Station, Texas (February, 1974).

In January, 1975, he continued teaching at the Universidad Nacional de Colombia in Bogotá. He taught five courses in veterinary toxicology and four courses in neurophysiology to veterinary and animal science students between February, 1975, and December, 1976. In September, 1975, he became Professor in Charge of Physiology of the Graduate School (Universidad Nacional de Colombia and Instituto Colombiano Agropecuario) and taught two courses in physiology. In August, 1975, he organized and participated in the 50 hour course, "Health Effects and Environmental Toxicology," under Dr. George T. Edds in Bogotá.

In February, 1976, he was invited by the American College of Veterinary Toxicologists and the University of Florida to lecture on "Blood-Body Fluids and Serum Enzymes" at the Third Veterinary Toxicology Symposium, Gainesville. During this month, he taught the "Short Course of Veterinary Toxicology" for Spanish-speaking veterinarians at the University of Miami,

Miami, Florida. He also translated from English to Spanish, the National and State Board Examination for Spanish-speaking veterinarians in May, 1976, and June, 1979.

While he was in Bogotá, he carried out research work on "Aflatoxins in Colombia," "Oil and Noise Toxicity," "Effects of the Organophosphate Insecticides in the Bull's Spermatogenesis." He also supervised research work on "Pollution of the Bogotá River and Pathological Effects in Fishes," "Chronic and Reproductive Toxic Effects of Thrichloform in Mice," "Toxic and Pharmacological Effects of the Mata de Tinto (Solanum chamaecerasus Bitter)."

In February, 1977, he joined the 3 year program with the University of Florida as a Graduate Research Assistant. working on a University/EPA grant of $800,000, "Health Effect of Urban Sewage Sludge." Special emphasis included acute and chronic toxicity studies, clinical chemistry, hematologic, electrophoretic, parasite and pathologic evaluations, metal residue effects, experimental design, data processing and statistical analyses.

He attended the 50 hour course in "Pharmacokinetics and Biopharmaceutics" under Dr. Edward R. Garrett, distinguished Professor of Pharmacokinetics, University of Florida, St. Augustine, Florida, April, 1977.

He presented the paper, "Feeding Trials of Dried Urban Sludge and the Equivalent Cadmium Level in Swine," at the 8th National Conference and Exhibition on Municipal Sludge Management, Miami Beach, Florida, March, 1979.

He entered the graduate program again to pursue the Ph.D. degree in March, 1977. His major degree in toxicology and pharmacology and his minor in neuroscience involved inter-departmental training in the Departments of Pharmacology and Therapeutics, Neuroscience, College of Medicine and Department of Preventive Medicine, College of Veterinary Medicine.

He is a Fellow of the American College of Veterinary Toxicologists and a member of the Veterinary Medical Association of Colombia.

Orlando Osuna is married to the former Clara Eugenia Navarro and is the father of one 8 year-old child, Diego Andrés.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

George T. Edds, Chairman
Professor of Toxicology
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Franklin H. White
Professor of Veterinary Medicine

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John W. Harvey
Professor of Veterinary Medicine

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Floyd J. Thompson

Floyd J. Thompson
Professor of Veterinary Medicine

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Ray L. Shirley

Ray L. Shirley
Professor of Animal Science

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1979

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